Dinoflagellate Cysts and *Chattonella* Resting Stages from Recent Sediments of the Southeast Coast of Iran

by

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In The Name of God,

The Compassion and The Merciful

DECLARATION

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ABSTRACT

Many harmful microalgae, including dinoflagellates and raphidophytes, produce long-lived, resistant, resting stages called resting cysts. Mapping the presence and abundance of these stages in coastal sediments combined with germination studies can provide valuable information on the distribution of potentially harmful species in an area. The resting cyst flora of equatorial and tropical regions of the world is poorly known, particularly in coastal waters. This thesis examines the dinoflagellate resting cyst flora in coastal marine sediments collected along the southern coast of Iran. The aim of the study was twofold. Firstly, to determine whether potentially harmful dinoflagellates or raphidophytes are present in the area. Secondly, to document the species diversity of dinoflagellate resting cysts in a little studied tropical region.

Nine sediment samples were collected at three locations (i.e. the Bahoo-kalat estuary, Pasabandar coast and Chabahar Bay) along the southeast coast of Iran. Dinoflagellate cyst species abundance and diversity was examined by light and scanning electron microscopy, and the resting cyst identity and affinity confirmed by cyst germination, where possible. Established cultures were further characterised by PCR amplification and DNA sequencing of the large subunit ribosomal RNA gene (LSU-rDNA) and internal transcribed spacer (rDNA-ITS) regions.

Over forty (40) distinct cysts morphotypes were identified in the samples examined. The most common groups identified were: *Scrippsiella* species (79%), *Protoperidinium* species (10%) and, at three sites (Pasabandar) an unusual angular, trapeziform resting cyst was common (7%). Potentially harmful species were also found in some sites; for example, the paralytic shellfish poisoning (PSP) causative species, *Alexandrium tamarense*, yessotoxin producing species *Lingulodinium polyedrum.*

Cyst incubation and germination studies established over 30 dinoflagellate cultures that were further characterised by detailed morphological (LM and SEM) and molecular approaches. The unusual trapeziform resting cysts were successfully germinated and identified as a previously undescribed dinoflagellate. Morphological studies of the resting cysts showed a microreticulate paratabulation, with the pattern reflecting amphiesmal patterning of the motile cell, including the cingulum and sulcus. Using cultures established from cyst germination the phylogenetic affinities of the species was examined by comparison of partial LSU-rDNA sequences with a range of other Gymnodinioids. The resulting analyses indicated a clear relationship to the other three known microreticulate cyst-forming Gymnodinoid species, *Gymnodinium catenatum*, *G. nolleri* and *G. microreticulatum*. Identical resting cysts were also successfully produced in nutrient depleted laboratory cultures, and the pattern of successful crosses indicated a homothallic mating system for this species. This dinoflagellate is described as a new species *G. trapeziforme* Attaran-Fariman & Bolch sp. nov.

The majority of the remaining cultures established from single cyst incubations were found to be allied with the Calciodinelloidean genera *Scrippsiella*, *Calciognellum*, *Calciodinellum*. Of these cultures, four distinct morphotypes were identified and

subjected to DNA sequencing of the rDNA-ITS regions. Three morphotypes were allied with *Scrippsiella trochoidea* var. *aciculifera*, *Scrippsiella* sp., and *Scrippsiella trochoidea* respectively. The fourth morphotype, possessing a small second anterior intercalary plate, was allied with but distinct from the similarly tabulated *Scrippsiella precaria* and *S. ramonii*. Morphological examination shows that its overall size and shape is similar to *S. precaria*, but with a more equatorially placed cingulum, an antapically placed nucleus and a larger, rounded 2nd anterior intercalary plate similar to *S. ramonii*. This species is described here as *Scrippsiella irregularis* Attaran-Fariman & Bolch sp. nov.

Mixed sediment incubations from site 6 (Pasabandar coast) also commonly released a raphidophyte belonging to the genus *Chattonella* Biecheler. Uni-algal cultures established from incubations were examined and determined to be allied with *Chattonella subsalsa* Biecheler. Analysis of the LSU-rDNA and rDNA-ITS regions sequences compared to other known *Chattonella* species showed that this species is related to *C. subsalsa*, but is genetically distinct, and may represent a new species.

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CHAPTER 1

Introduction

1. General Introduction

Dinoflagellates are an important group of microscopic unicellular algae that are a dominant group of the phytoplankton (Taylor 1987). Among 2000 extant dinoflagellate species, 60 species are known to produce toxins (Steidinger & Tangen 1997). They also have an essential role as a primary producer in food chain and are a vital food for filter feeding shellfish such as oysters, clams and scallops (Raine 2002).

Dinoflagellates possess two flagella: one transverse flagellum that encircles the body in a horizontal groove-like structure (cingulum or girdle), providing forward movement and spin to the dinoflagellate; the second is a longitudinal flagellum positioned in a vertical groove, from the mid-ventral area toward the antapex, termed the sulcus. The cell wall of the dinoflagellate may have a clear arrangement of cellulose plates called a theca (armoured), or the absence of a rigid outer cell covering, termed athecate or unarmoured. Dinoflagellates have variety of life/feeding strategies, from autotrophic (photosynthetic), heterotrophic (non-photosynthetic), photographic or mixotrophic to symbiosis or parasitism (Taylor 1987).

Dense concentrations (up to millions of cells per litre) of phytoplankton are known as algal blooms which, discolour the water changing it to yellow, orange, brown purple or red (i.e. called "red tide") depending on the species and the concentration of the cells. Favourable growth conditions cause rapid increase in the numbers of

phytoplankton cells (Franklin *et al.* 2004). Most non-toxic algal blooms are natural phenomena and completely harmless, but sometimes, if very densely concentrated, they may cause anoxia (oxygen depletion) or damage to fish gills and also damage to other invertebrates, resulting in mortality of fish and other marine organisms.

Blooms of toxic species, even at low concentrations can kill or contaminate shellfish by producing a toxin (Hallegraeff 1993). Toxins also can be filtered from water by shellfish, such as clams, mussels, oysters and scallops or consumed by fish. The shellfish or fish then act as transitional vectors, collecting the algal toxin to high levels, and passing it to consumers at higher levels. Some of these toxins are neurotoxic such as Neurotoxic Shellfish Poisoning (NSP) (Holmes & Teo 2002; Holmes *et al.* 2002). A second group of toxins are powerful paralytic toxins such as the well known Paralytic Shellfish Poisoning (PSP) (Kirkpatrick *et al.* 2004; Ishida *et al.* 2004). Some other shellfish and fish poisoning syndroms are: Diarrheic Shellfish Poisoning (DSP) (Picher & Calder 2000), Amnesic Shellfish Poisoning (ASP) (Trainer 2000), Azaspiracid Shellfish Poisoning (AZP) and also Ciguatera Fish food Poisoning (CFP) (Ochoa *et al.* 1997; James *et al.* 2002).

Dinoflagellates are important bloom formers in all coastal waters, freshwater and lake ecosystems. Almost 75% of all harmful algal bloom (HAB) species are dinoflagellates (Keafer *et al.* 1992; Smayda 1997). Many dinoflagellates have been reported as spending periods of their life cycle resting in the sediments. These resting stages are called cysts. Dinoflagellate cysts are resting cells formed during the process of sexual reproduction that can survive in unfavourable conditions and stay dormant, waiting for better environmental conditions to germinate (Matsouka & Fukuyo 2000). The cyst population acts as the "seed bank" for stabilising the planktonic population (Lewis *et al.* 1999). Therefore, alternation between planktonic life stage in the water and resting stage in the sediment is one of the central factors determining the occurrence, timing and development of HABs (Dale & Montresor 2001). At least 260 dinoflagellate species produce resting cysts (Head 1996), including many harmful species that cause red tides.

1.1. History of Dinoflagellate Cyst Studies

The history of the study of fossil and living dinoflagellate cysts has been reviewed by Evitt (1964), Sarjeant (1974) and Dale (1983). Their findings are summarised as follows. The first fossil cysts were seen by Ehrenberg in the 1838 in thin flakes of German Cretaceous flint. As he had only studied living plankton, previously he attributed some of these microfossils to the living genus *Peridinium.* Ehrenberg observed microfossils of similar size to the specimens with variable patterns of spines in the same flint and identified them as dinoflagellates. He assumed that these silicified spiny body fossil forms were comparable to the zygospore of green fresh water algae and termed them "*Xanthidia*". The first generic name for dinoflagellates was "*Actiniscus*" described by Ehrenberg in 1840. Initially he suggested that they were a group of *Dictyocha* (now known as silicoflagellates), but in 1843 they were given a full generic position. In 1845, Mantell concluded that "*Xanthidia*" were not silicified and suggested such fossils were probably organic and in 1850 named these spinous fossils as "*Spiniferites*". A new genus (*Hystrichosphaera*) was proposed for fossil dinoflagellates (or *Xanthidia*) and this became a popular name for spiny microfossils (Sarjeant 1974).

From the early 1930s onwards, palaeontological research on cysts began to develop techniques for chemical extraction of fossils from rock and the study of these microfossils in stratigraphy and palynology developed greatly. However, biologists rarely record cysts as a part of plankton surveys and very few studies on living cysts were comparable to those done on fossils.

Huber and Nipkow (1922) documented encystment of the fresh water species *Ceratium hirundinella* Bergh both in lakes and cultures. Later, encystment of the marine species, *Alexandrium tamarense* Lebour (Balech) and *Protoceratium reticulatum* (Claparede & Lachmann) reported by Braarud (1945).

Sporadic records of Hystrichospheres in modern sediment continued but the true affinity of *Hystrichospheres* remained unknown, until Evitt's observations in 1961. He recognised basic morphological features of many *Hystrichospheres* and the presence of a characteristic opening on the walls of cysts that he called archeopyle.

He also found spines or processes on cysts that reflected a typical plate pattern on a dinoflagellate. Subsequently Evitt and Davidson (1964) described cyst-theca relationships.

Later, Wall and Dale (1968) conducted a series of germination studies of cyst species from bottom sediment and sea water documenting many cyst-theca relationships. From this time onward, studies on different aspects of modern dinoflagellate increased rapidly. Over the past decades, many studies have been conducted to represent dinoflagellate cysts and their distribution in different areas (e.g. Bolch & Hallegraeff 1990; Vink *et al.* 2000; Zonneveld *et al.* 2000; Cho & Matsouka 2001; Wang *et al.* 2004).

1.2. Taxonomy and Classification of Dinoflagellate Cysts

The taxonomy of dinoflagellate cysts is based on two different systems. Palaeontologists have developed a palaeontological classification based on cyst features, whereas phycologists classified them based on motile cells. For example, Wall and Dale (1968) found modern cysts of *Gonyaulax digitalis* (Pouchet) Kofoid with the same morphology as the fossil species *Hystrichosphaera nodosa*. These modern cysts have also been classified and described under the palaeontological system. As each taxonomic system for classification is different, it is not surprising that one species sometimes has more than one scientific name. Some common dinoflagellate resting cysts are summarised in Table 1.

Fensome *et al.* (1993) produced a unified classification of living motile dinoflagellates and fossil dinoflagellates with the emphasis on tabulation and paratabulation. This work recognised six major tabulation types: gymnodinioid, suessioid, gonyaulacoid-peridinoid, dinophysioid, nannoceratopsioid and prorocentroid. In armoured dinoflagellates, thecal plate patterns are the key morphological characteristic, but in the case of unarmoured dinoflagellates, the structure/shape of chloroplasts, flagellar roots and cell contents are important to classify them (Hallegraeff 1995a). However, almost every important feature varies markedly from species to species and across genera.

Since the work of Wall and Dale in 1968 on cyst/theca correlation, many studies of cyst-theca relationships have followed by cyst germination experiments (e.g. Bolch & Hallegraeff 1990; Lewis 1991; Ellegaard *et al.* 2002; Lewis *et al.* 2001) and provided a more accurate taxonomic and ecological understanding of dinoflagellates. Despite the increasing studies over the past decades, only some marine dinoflagellates have been correlated to their resting cysts.

Morphological characteristics and biogeographical distributions of organisms have been considered as a primary means for traditional species classification (Hallegraeff 1995). Recently, applications combining genetic and morphological criteria have become increasingly important for the characterisation, separation, and identification of dinoflagellate cyst species.

Table 1: List of some common dinoflagellate resting cyst species in recent sediments (Modified from Head 1996; Matsouka & Fukuyo 2000; recent journal articles)

Cyst Species Biological Name	Cyst Species Palaeontological Name	References
Peridiniales		
Scrippsiella crystallina		Lewis (1991), Montresor *et al.* (1993)
Scrippsiella lachrymosa		Lewis (1991), Nehring (1994)
Scrippsiella hangoei		Larsen *et al.* (1995)
Scrippsiella minima		Gao *et al.* (1989), Gao & Dodge (1991)
Scrippsiella pentagonica	*Obliquipithonella irregularis*	Akselman & Keupp (1990)
Scrippsiella precaria		Montresor & Zingone (1988)
Scrippsiella ramonii		Montresor (1995)
Scrippsiella regalis	*Rhabdothorax gerenus*	Janofske (2000)
Scrippsiella rotunda		Lewis (1991)
Scrippsiella trifida		Lewis (1991)
Scrippsiella trochoidea	*Rhabdothorax erinaceus*	Dodge (1982), Bolch & Hallegraeff (1990), Janofske (2000)

Table 1 ***Continued...***

Scrippsiella sweeneyae	*Wallidinellum dalei*	Wall & Dale (1968)
Ensiculifera cf. mexicana	*Pentadinellum oblatum*	Wall and Dale (1968)
Ensiculifera carinata		Matsuoka *et al.* (1990)
Ensiculifera imariensis		Kobayashi & Matsuoka (1995)
Pentapharsodinium dalei		Dale (1977); Lewis (1991)
Pentapharsodinium tyrrhenicum		Montresor *et al.* (1993)
Pernambugia tuberosa		Karwath *et al.* (2000); Gottschling *et al.* (2005)
Calciodinellum infula		Fensome *et al.* (1993); Gottschling *et al.* (2005)
Calciodinellum albatrosianum		Gottschling *et al.* (2005)
Calciodinellum levantinum		Gottschling *et al.* (2005)
Calciodinellum operosum		Vink (2004); Gottschling *et al.* (2005)
Heterocapsa niei		Head (1996)
Heterocapsa triquetra		Steidinger & Tangen (1996)
Pfiesteria piscicida		Burkholder & Glasgow (2002); Parrow & Burkholder (2004)
Pfiesteria shumwayae		Burkholder *et al.* (2001)
Protoperidinium americanum		Lewis *et al.* (1984); Bolch & Hallegraeff (1990)
Protoperidinium avellana	*Brigantedinium cariacoense*	Wall & Dale (1968); Lewis *et al.* (1984)
Protoperidinium antarcticum		McMinn & Scott (2005)
Protoperidinium brochii		Head (1996)
Protoperidinium claudicans	*Votadinium spinosum*	Wall & Dale (1968)
Protoperidinium compressum	*Stelladinium reidii*	Wall & Dale (1968)
Protoperidinium conicoides	*Brigantedinium simplex*	Wall & Dale (1968)
Protoperidinium conicum	*Selenopemphix quanta*	Bolch & Hallegraeff (1990)
Protoperidinium denticulatum	*Brigantedinium irregulare*	Wall & Dale (1968)
Protoperidinium divaricatum	*Xandarodinium xanthum*	Matsuoka *et al.* (1982)
Protoperidinium excentricum		Wall & Dale (1968); Lewis *et al.* (1984)
Protoperidinium expansum		Hallegraeff & Bolch (1992)

Table 1 ***Continued...***

Protoperidinium gainii		Head (1996)
Protoperidinium latissimum		Wall & Dale (1968)
Protoperidinium leonis	*Quinquecuspis concreta*	Dodge (1985)
Protoperidinium minutum		Wall & Dale (1968)
Protoperidinium monospinum		Zonneveld & Dale (1994)
Protoperidinium nudum	*Multispinula quanta*	Wall & Dale (1968); Bradford (1975)
Protoperidinium oblongum	*Votadinium calvum*	Wall & Dale (1968); Reid (1977)
Protoperidinium obtsum		Matsuoka & Fukuyo (2000)
Protoperidinium pentagonum	*Trinovantedinium capitatum*	Wall & Dale (1968); Matsuoka *et al.* (1982)
Protoperidinium punctulatum	*Brigantedinium cariacoense*	Wall & Dale (1968)
Protoperidinium subinerme	*Selenopemphix alticinctum*	Bradford (1975); Matsuoka *et al.* (1982); Matsuoka (1985)
Protoperidinium thorianum	*Brigantedinium* sp.	Lewis *et al.* (1984); Matsuoka (1992)
Protoperidinium thulesense		Dodge (1985)
Protoperidinium cf. *divergens*	*Peridinium ponticum*	Dale (1983); Nehring (1994)
Diplopelta parva		Bolch & Hallegraeff (1990); Dale *et al.* (1993)
Diplopelta symmetrica		Wall & Dale (1968)
Diplopsalis lenticula		Dale (1983); Matsuoka (1988)
Diplopsalopsis orbicularis	*Duridinium cavatum*	Dale *et al.* (1993)
Diplopsalopsis latipeltata		Matsuoka (1988)
Zygabikodinium lenticulatum		Bolch & Hallegraeff (1990)
Gymnodiniales		
Cochlodinium sp.		Matsuoka (1985)
Gymnodinium catenatum		Bravo (1986); Anderson *et al.* (1988)
Gymnodinium nolleri		Ellegaard & Oshima (1998)
Gymnodinium microreticulatum		Bolch *et al.* (1999)
Gymnodinium impudicum		Daugbjerg *et al.* (2000); Gasol *et al.* (2005)
Gymnodinium instriatum		Daugbjerg *et al.* (2000)
Gyrodinium resplendens		Hulburt (1957); Skovgaard (2000)

Table 1 ***Continued...***

Gyrodinium uncatenum		Anderson *et al.* (1988); Cetta & Anderson (1990)
Gymnodinium aureolum		Hansen *et al.* (2000); Daugbjerg *et al.* (2000)
Pheopolykrikos hartmannii		Matsuoka & Fukuyo (1986)
Polykrikos kofoidii		Nagai *et al.* (2002)
Polykrikos schwartzii		Wall & Dale (1968); Matsuoka (1985)
Woloszynskia sp. 1		Bolch & Hallegraeff (1990)
Katodinium fungiforme		Dodge (1982)
Warnowia cf. *rosea*		Ellegaard *et al.* (2002)
Gonyaulacales		
Gonyaulax digitalis	*Spiniferites bentorii*	Wall & Dale (1968); Ellegaard *et al.* (2003)
Gonyaulax baltica	*Spiniferites bulloideus*	Wall & Dale (1968); Ellegaard *et al.* (2002)
Gonyaulax elongata	*Spiniferites elongatus*	Reid (1974); Ellegaard *et al.* (2003)
Gonyaulax membranacea	*Spiniferites membranaceus*	Reid (1974); Ellegaard *et al.* (2003)
Gonyaulax spinifera	*Spiniferites mirabilis*	Dodge (1985); Bolch & Hallegraeff (1990)
Gonyaulax spinifera	*Spiniferites ramosus*	Bradford & Wall (1984); Lewis *et al.* (1999)
Gonyaulax spinifera complex	*Nematoshaeropsis lemniscata*	Dodge (1985); Matsuoka (1992)
Gonyaulax spinifera complex	*Spiniferites frigidus*	Matsuoka (1992)
Gonyaulax verior		Matsuoka & Fukuyo (1988)
Lingulodinium polyedrum	*Lingulodinium machaerophorum*	Lewis (1988)
Protoceratium reticulatum	*Operculodinium centrocarpum*	Bolch & Hallegraeff (1990); Sonneman & Hill (1997)
Alexandrium affine		Fukuyo (1985); Band-Schmidt *et al.* (2003)
Alexandrium andersoni		Montresor *et al.* (1998)
Alexandrium catenella		Hallegraeff *et al.* (1991); Sonneman & Hill (1997)
Alexandrium cohorticula		Ogata *et al.* (1990)
Alexandrium fundyense		Haya *et al.* (2003)
Alexandrium hiranoi		Hallegraeff (2002)
Alexandrium leei		Matsuoka & Fukuyo (2000)

Table 1 ***Continued...***

Coolia monotis		Faust (1992)
Alexandrium margalefi		Hallegraeff *et al.* (1991)
Alexandrium minutum		Bolch *et al.* (1991), Hallegraeff *et al.* (1991).
Alexandrium monilatum		Hallegraeff *et al.* (1991).
Alexandrium ostenfeldii		Faust & Gulledge (2002)
Alexandrium peruvianum		Matsouka (1992)
Alexandrium pseudogonyaulax		Montresor & Marino (1996)
Alexandrium tamarense		Oshima *et al.* (1992)
Alexandrium taylori		Garces *et al.* (1998); Giacobbe & Yang (1999)
Fragilidium subglobosum		Sonneman & Hill (1997)
Fragilidium heterolobum		Head (1996)
Pyrodinium bahamense		
var. bahamense	*Polysphaeridium zoharyi*	Bradford & Wall (1984); Sombrito *et al.* (2004)
var. compressum	*Polysphaeridium zoharyi*	Bradford & Wall (1984)
Pyrophacus horologium		Head (1996)
Pyrophacus steinii	*Tuberculodinium vancampoae*	Faust (1998)

2. Cyst Morphology

In the life cycle of a dinoflagellate, the cyst and motile stages may be considered as two different morphological expression of one set of genes (Dale 1983). These morphological differences reflect the different functions of the motile and non-motile stages. There are wide ranges of morphological overlaps between these two (Fig. 1) that allow us to recognise cyst and motile stages (for example: paracingulum on the cyst and cingulum on the motile stage). In the cyst, the same terminologies are used as the motile stage, but are expressed with the prefix 'para' (Evitt *et al.* 1977). Therefore, parasulcus, paracingulum, paratabulation, and paraplate are used for cyst morphology. Cyst morphology is distinctive within a species and is generally less

conservative than morphology of the motile stage (Sarjeant 1974), therefore cysts are classified by their morphological features. The important morphological characteristics for classification of cysts are: the shape of the body, cyst wall structure and colour, type of ornaments, the type of archeopyle, paratabulation and the cyst contents (Anderson *et al.* 1995).

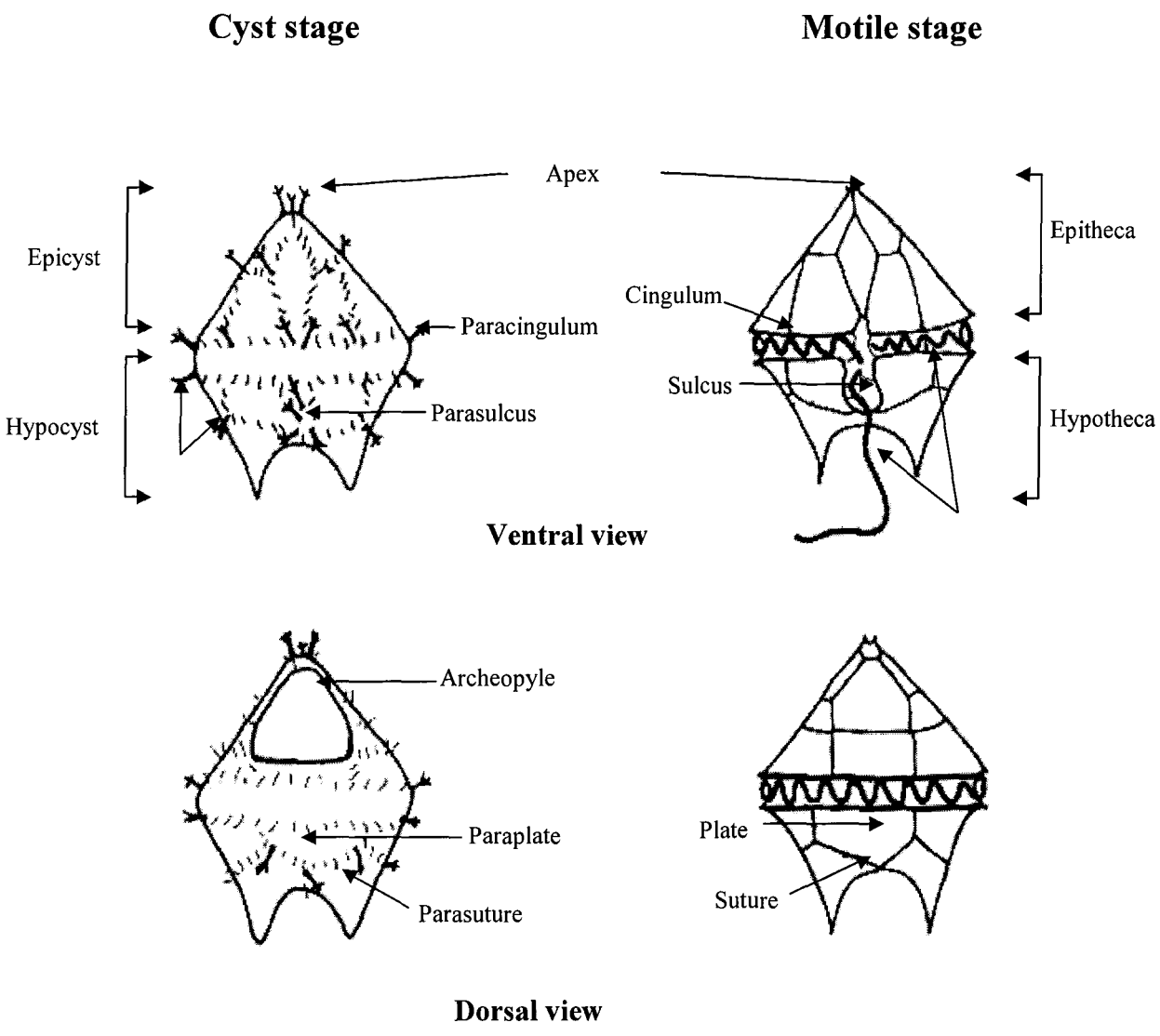


Fig. 1. Main features of cyst and motile stage (After Dale 1983)

2.1. Overall Body Shape of Cysts

The body shape of dinoflagellate cysts may vary from species to species, but it remains generally constant within a species. However, sometimes environmental factors (such as salinity, temperature and nutrients) can produce remarkable variation in the cyst shape of a single species (Matsuoka & Fukuyo 2000). Cyst bodies vary from spherical to ovoidal, subspherical, cylindrical and peridinoid (e.g. *Protoperidinium* spp.). Some cysts vary in shape from sub-rectangular to trapezoidal. The position and type of horns (which may be curved or pointed, e.g. *Protoperidinium* spp.), paracingulum and parasulcus are also important features in classifying cysts. *Gonyaulax digitalis* (Pouchet) Kofoid shows expression of paratabulation on the cyst.

2.2. Cyst Wall Structure and Colour

The wall of dinoflagellate cysts shows variation between species. The cyst wall contains one, two or three layers called the periphragm, mesophragm, and endophragm (Matsuoka & Fukuyo 1995). The wall may be organic or mineral walled. Organic cyst walls are resistant to both natural decay and laboratory acid treatment. Many cysts are extremely acid-resistant and capable of fossilisation because of sporopollen-like material in the wall, or less acid resistant and can not be fossilised (Dale 1983). Dale (1983) also described the less resistant *Protoperidinium* cysts as usually a distinct light to dark brown colour, whereas sporopollenin-like cysts are mostly colourless.

Some cysts have a mineral wall that is destroyed by hydrochloric acid (Hcl) and hydrofluoric acid (HF). Fossil calcareous cysts from the Quaternary and living calcareous cysts from bottom sediments have been described by Wall and Dale (1968). *Scrippsiella trochoidea* produces calcareous cysts. *Ensiculifera* also produces calcareous cysts and the inner walls of these cysts are thin and organic acid resistant (Dale 1983). The colour of the cyst wall is also variable. It may be transparent and colourless (e.g. *Spiniferites mirabilis* (Rossignol) Sarjeant), yellow, purple, or light to dark brown (e.g. *Protoperidinium* spp.).

2.2.1. Cyst Wall Processes

The surface of the cyst may be smooth (as are some round brown cysts of *Protoperidinium*) or ornamented by different types of processes and projections (e.g. *Gonyaulax* spp.). The type of process is usually solid or hollow, flat tipped, flared or pointed (Fig. 2). The terms and descriptions for processes and morphology of dinoflagellates cysts were introduced by Sarjeant (1984) and modified by Matsuoka and Fukuyo (2000). The latter defined three major morphological groups in dinoflagellate cysts based on process length as a percentage of the shortest diameter of the central body. These are proximate cysts, proximochorate cysts and chorate cysts (Fig. 3). The process length, however, may vary considerably in some species within a population. For example, the cyst of *Lingulodinium polyedrum* (Stein) Dodge (= *Gonyaulax polyedra* Stein) often have shorter processes in very low salinity (Wall *et al.* 1973).

Therefore, these three categories based on process length are not particularly stable characters in many species and their taxonomic value is questionable.

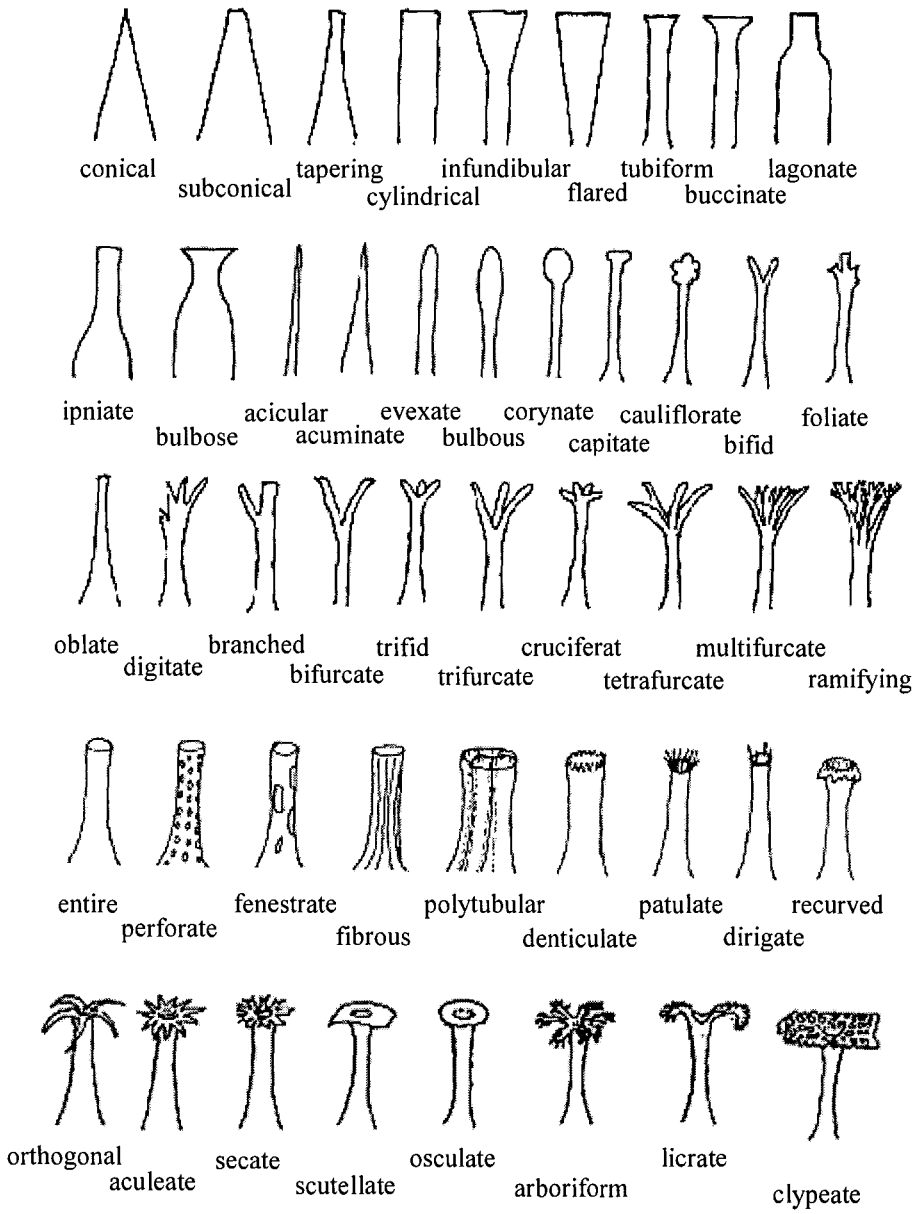


Fig. 2. The morphological terms for dinoflagellate cysts processes (After Matsouka & Fukuyo 1995)

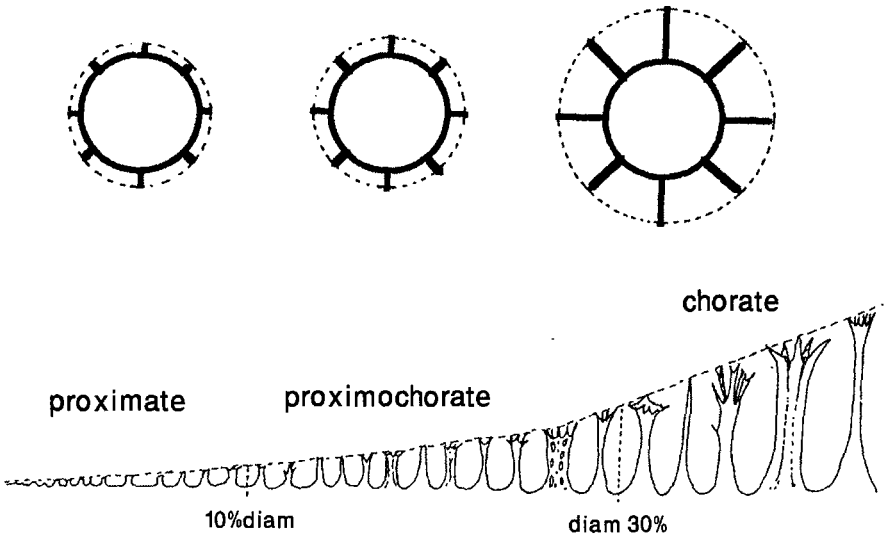


Fig. 3. Different type of dinoflagellate cyst based on processes length (After Matsouka & Fukuyo 2000)

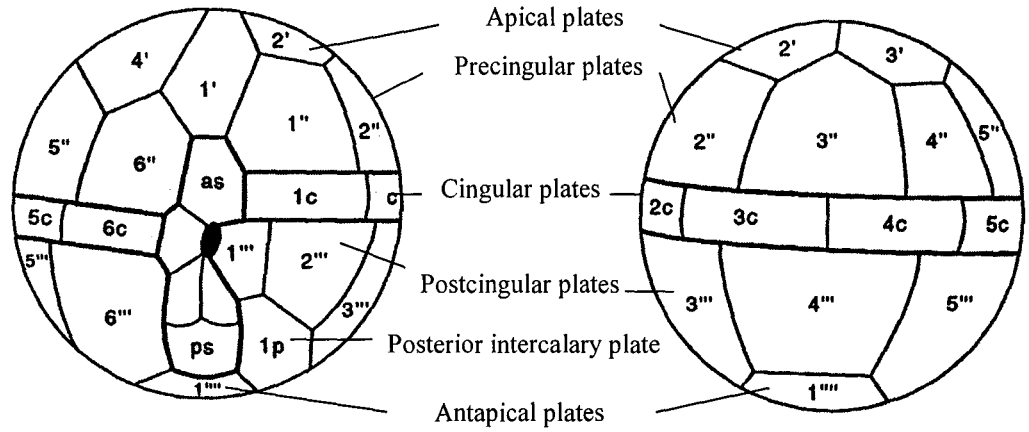
2.2.2. Cyst Wall Paratabulation

Paratabulation is the tabulation or plate pattern of the motile cell reflected on the cysts. Many features of the cyst such as processes, paracingulum, parasulcus, archeopyle (excystment opening) and paravesicle patterns correspond to features of the motile stages. Some cysts show remarkable resemblance to thecae, such as *Zygabikodinium lenticulatum* (Mangin) Loeblich & Loeblich III, where the expression of the paracingulum and parasulcus on the cyst wall is clearly visible. Paratabulation is well illustrated in the fossil record. For example, the one process-one plate relationship was also illustrated for spiny cyst of *Hystrichosphaeridium* by Evitt (1985). In addition, the tabulation of living *L. polyedrum* corresponds to the fossil proximate cyst of *Gonyaulacysta jurassica* (Deflandre) Sarjeant (Sarjeant 1974).

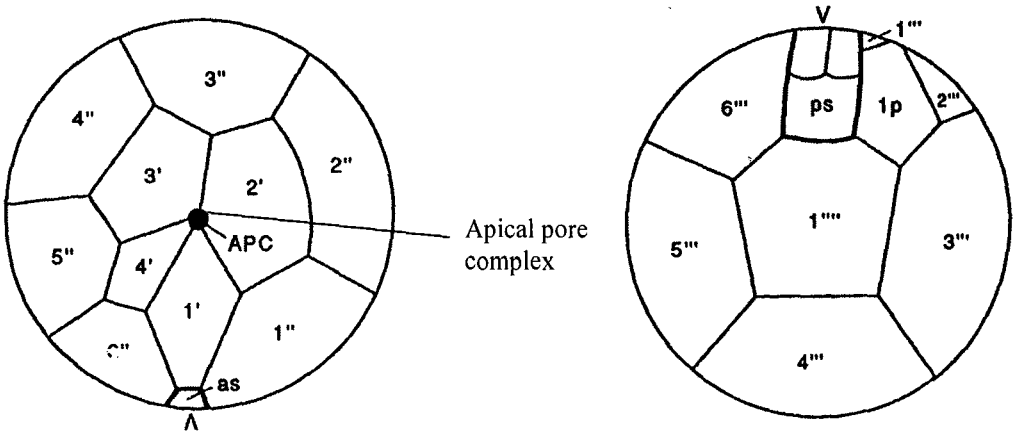
Two groups of dinoflagellate have been described in the literature on the basis of cell wall and tabulation appearance. Dinoflagellates possess a complex and structurally variable cell covering or amphiesma (Fensome *et al.* 1993). The amphiesma has a

layer of vesicles that can be numerous (up to several thousand per cell) with a random arrangement, or can be a few vesicles. These vesicles may or may not contain cellulosic plates. Those dinoflagellates in which the amphiesmal vesicles have cellulosic plates are called thecate or armoured, and those without plates are called athecate or naked (Fensome *et al.* 1993). Naked dinoflagellate cells are surrounded by a thin, highly flexible protein pellicle that consists of a very thin outer layer with a flat vesicle and a thicker inner layer (Sarjeant 1974). The vesicle pattern can be seen under light microscopy, only after special staining, or under electron microscopy. Armoured dinoflagellates show a clear tabulation when examined under light microscopy (fluorescence microscopy). Tabulation and vesicle patterns are the most reliable criteria in the taxonomy of dinoflagellates (Fensome, *et al.* 1993).

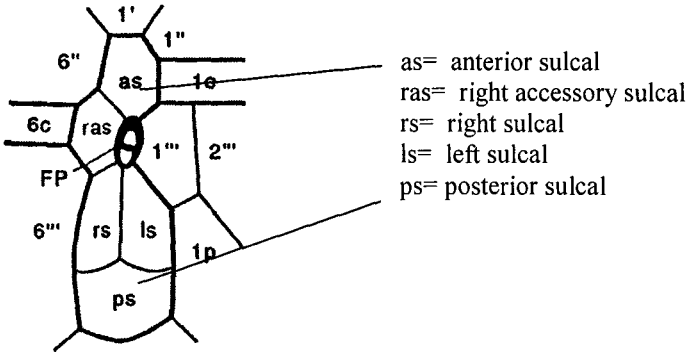
There are several plate labelling (tabulation) models (e.g. Eaton tabulation model or Edward's tabulation model), but the most commonly used is the Kofoid tabulation system (Fig. 4) (Fensome *et al.* 1993). In this system five parallel series of plates are defined; apical plates, precingular plates, postcingular plates, sulcal plates and antapical plates. Also, plates between the apical and precingular series and between antapical and postcingular series are called anterior and posterior intercalary plates respectively. The cysts of armoured dinoflagellates show features that correspond to the motile stage. With the exception of microreticulate cyst species (e.g. *Gymnodinium catenatum* Graham), most cysts of naked dinoflagellates show no paratabulation. General morphology and arrangement of amphiesmal vesicles in an athecate dinoflagellate species is shown in Fig. 5.



Ventral view Dorsal view

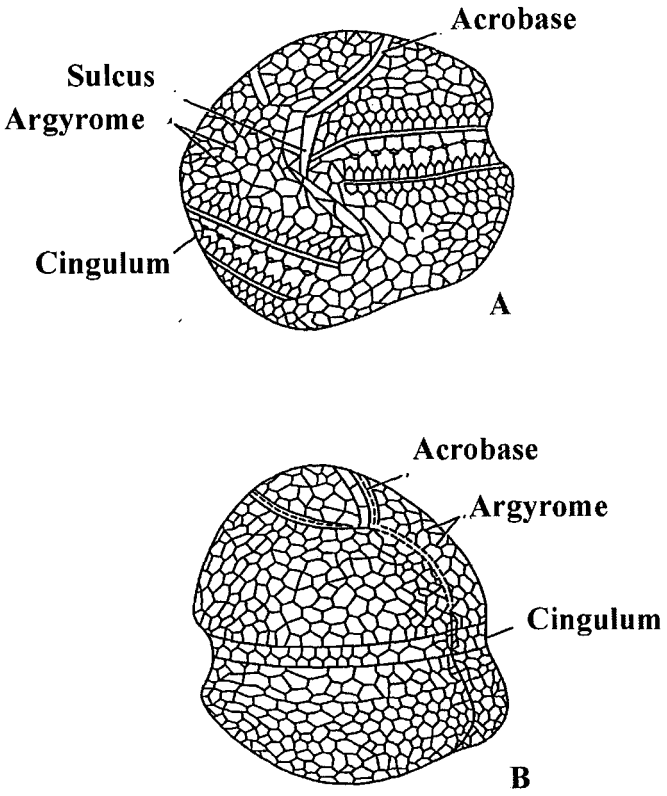


Apical view Antapical view



Sulcal tabulation

Fig. 4. Kofoid tabulation system in a thecate dinoflagellate (After Fensome *et al.* 1993) (Standard Gonyaulacalean tabulation as an example)



A = Ventral view of *Gyrodinium vorax*
B = Latroventral view of *Gymnodinium maguelonnense*

Fig. 5. General morphology of athecate dinoflagellates (Gymnodiniaceae) species (After Fensome *et al.* 1993)

2.3. Archeopyle

The type and position of the cyst opening, the archeopyle, is constant in any particular species (Matsouka & Fukuyo 2000). Therefore, shape and position of the archeopyle is one of the most important criteria in cyst taxonomy. The archeopyle was first defined by Evitt (1961) and five distinct types were later defined by Evitt and Davidson (1967). These types are: apical, intercalary, precingular, a combination of these series, and miscellaneous archeopyles. Miscellaneous archeopyles include cysts such as gymnodinialean species that do not have discernable plate patterns.

Matsouka (1985) proposed new descriptive terms for the archeopyles of modern dinoflagellate cysts for both armoured and unarmoured dinoflagellates and classified them into: saphopylic, theropylic and cryptopylic archeopyles (Fig. 6).

The shape of an arceopyle usually involves one paraplate (e.g. *Protoperidinium avellana* (Meunier) Balech) or more (e.g. *Protoperidinium denticulatum* (Gran & Braarud) Balech). According to Dale (1983), when one paraplate is involved, it is termed as operculum; if there is more than one paraplate with a suture between them, they are termed as opercular pieces. In some species the archeopyle sutures open completely, releasing the operculum or opercular pieces (e.g. *Protoperidinium oblongum* (Aurivillius) Parke & Dodge). In other species, it may remain partially attached.

2.4. Cell Contents

Live mature cysts often contain food storage products, such as starch grains or oil droplets. In some freshly formed cysts, or those ready to excyst, the microgranular cytoplasm may develop a quick Brownian motion like action, and cell contents are darker (Dale 1983). Some cysts include one or more obvious yellow to red pigment bodies of unknown function. These are termed eye spots or accumulation bodies. A fairly large nucleus is sometimes visible in cysts.

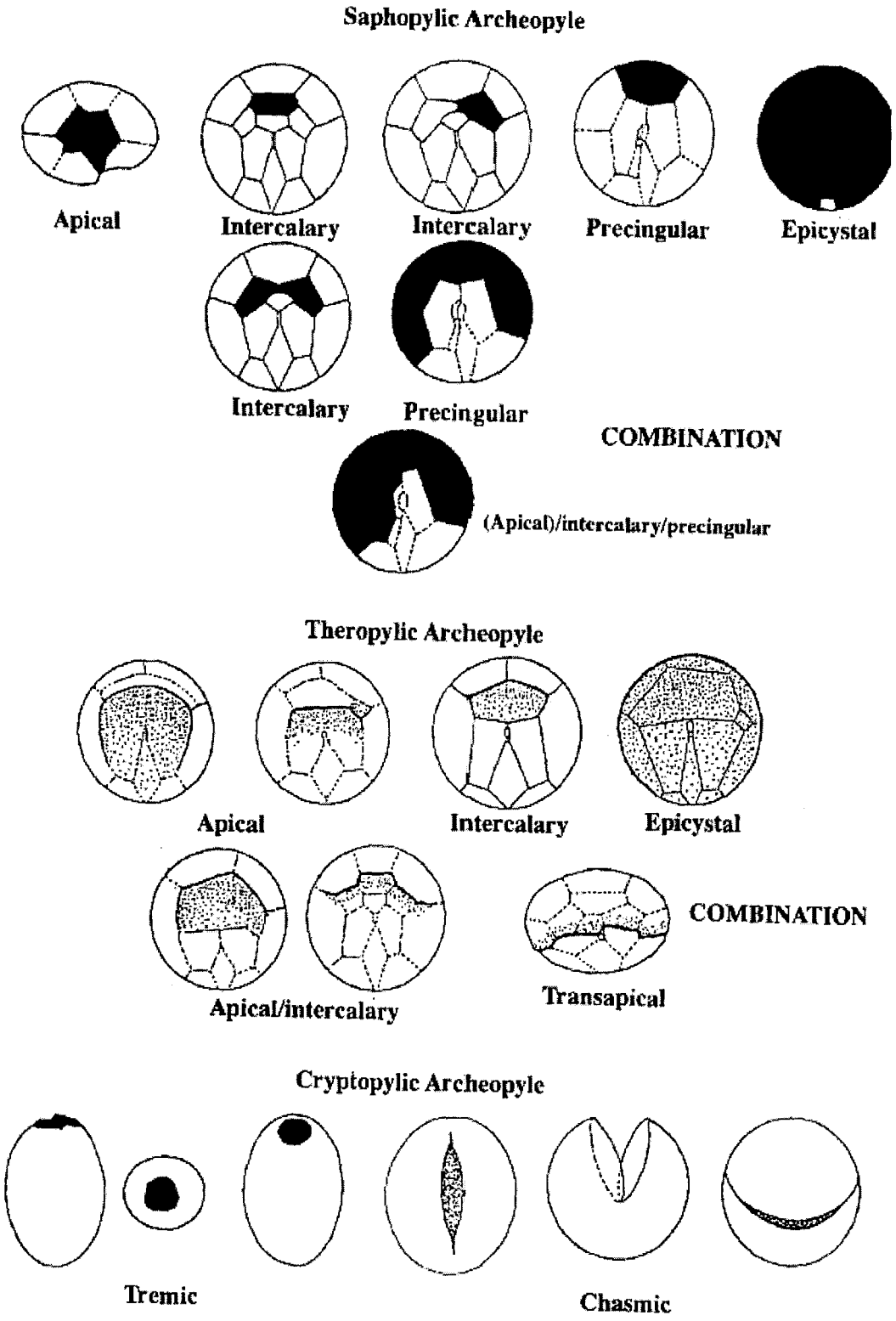


Fig. 6. Archeopyle types in recent dinoflagellate cysts (After Matsouka & Fukuyo 2000)

3. Molecular Approach to Study Dinoflagellate Cysts

Molecular data can provide useful information on such factors as phylogeny, geographical variation, and the level of genetic diversity or genetic structure of populations of coastal microalgae (Bolch *et al.* 1999).

Morphological characteristics of dinoflagellates are often difficult to interpret, because they can vary in response to changing environmental factors and culture conditions, so their validity for species level classification is questionable (Taylor & Fukuyo 1998). For example, Adachi *et al.* (1996), using rDNA sequence data, suggested that several *Alexandrium* species complexes are more closely related by geographical origin than by clear morphological features. In addition, Ellegaard *et al.* (2003) documented that cyst and cyst-based taxa of *Gonyaulax membranacea* (Rossignol) Sarjeant were morphologically different from those described by Lewis *et al.* (1999), but LSU rDNA sequence showed they were not genetically different species. Furthermore, LSU rDNA data showed that the sequence divergence between *Gonyaulax* species correlates with differences in cyst morphology rather than differences in motile cell morphology (Ellegaard *et al.* 2003). Therefore in cyst taxonomy studies it is important to combine studies of the morphology of cyst and motile stages with molecular approaches.

Several different molecular techniques have been used in genetic studies of dinoflagellates. Ribosomal RNA is a repeated multigene family, with each transcription unit containing three genes (Adachi *et al.* 1994). Sequence analysis of small-subunit (SSU) and large-subunit (LSU) ribosomal RNA (rRNA) genes (rDNA) is one method that can be used to assist classification of species. These genes are useful for analysing phylogenetic relationships in highly diverse taxa because of the presence of both highly conserved (LSU) and variable regions (ITS) (Penna & Magnani 1999). Therefore, sequences of rRNA and rDNA have been used widely to evaluate the evolutionary history of dinoflagellate and many other organisms (Scholin & Anderson 1994). Fensome *et al.* (1999) also pointed out that there are difficulties in the use of only molecular data in dinoflagellate classification and suggested that it must be compared with morphological data. Therefore, morphological characteristics, molecular analyses and phylogenetic relationships

between species, and combination of these approaches with both motile cells and cysts are important in the classification of dinoflagellates and other algae (e.g. Montresor *et al.* 2003; Ellegaard *et al.* 2003; Gottschling *et al.* 2005).

Some species, however, may not germinate or may be difficult to bring to pure culture, especially heterotrophic species such as *Protoperidinium*. species. For these species, Bolch (2001) suggested amplification of ribosomal RNA directly from single or small numbers of cysts or live cells.

4. Dinoflagellate Life Cycles

The life cycle of dinoflagellates is multi-staged, from simple to complex. They may include coccoid, cyst, multicellular or amoeboid stages. Dinoflagellates have asexual and sexual reproduction in their life cycle but the most common means of producing more dinoflagellates is simple asexual cell division (mitosis) (Fensome *et al.* 1993).

The life cycle of many dinoflagellates involves sexual reproduction with formation of a resting stage or cyst (Dale 1983). The occurrence of meiosis and sexual reproduction has been documented not only in armoured marine species (e.g. *Scrippsiella trochoidea* (Stein) Balech & Loeblich III, Uchida 1991) but also in unarmoured marine species (e.g. *G. catenatum*, Blackburn *et al.* 1989; Blackburn *et al.* 2001) and freshwater dinoflagellates (e.g. *Woloszynskia apiculata* von Stosch, von Stosch 1973).

A generalised dinoflagellate sexual life cycle is shown in Fig. 7. The sexual life cycle of dinoflagellates begins with gamete formation and then fusion of gametes to form a swimming zygote or planozygote. The planozygote has a common cingulum and a common sulcus, but two transverse flagella are still present so that it is differentiated from a normal motile cell (Figueroa & Bravo 2005a, 2005b). The planozygote loss motility after a few days transform into hypnozygote (resting cyst). After maturation time (dormancy), which is variable in length for different species, the cyst can germinate and produce a diploid zygote termed a "planomeiocyte", or remain as a quiescent stage in the sediments for many years. Following germination to release a

diploid planomeiocyte, a haploid, thecate, motile cell is produced by meiotic division.

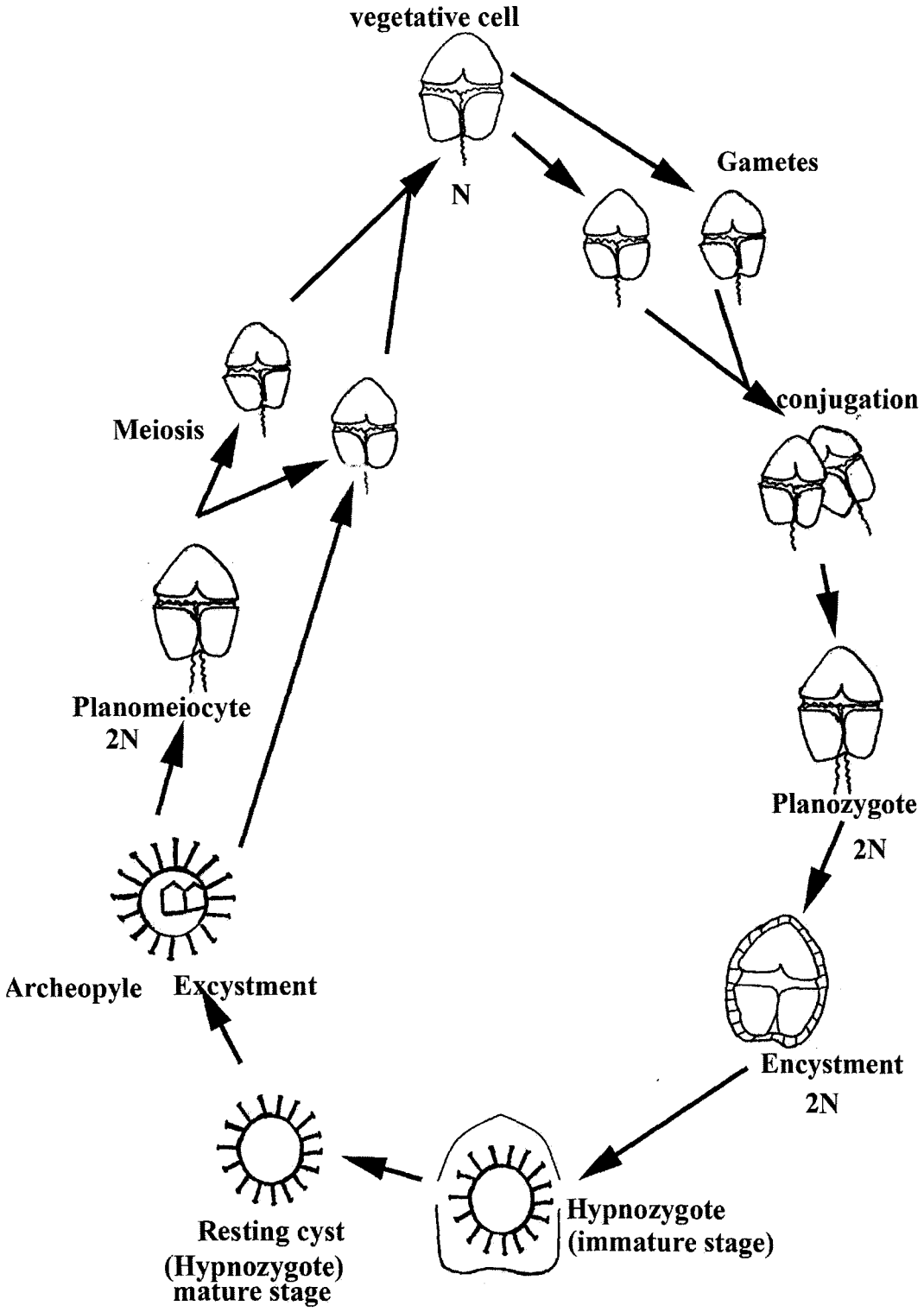


Fig. 7. A general sexual life cycle of dinoflagellates (after Dale 1983)

While cyst formation is a part of sexual reproduction, not all species include a clearly defined resting cyst, nor does the planozygote necessarily proceed to a cyst in all species (Montresor 2001). The planozygote can also produce two haploid motile cells by reduction division. The haploid motile cell or vegetative cell is capable of forming a temporary cyst or can produce two haploid daughter cells (Matsuoka & Fukuyo 2000).

4.1. Encystment

4.1.1. Cyst Formation

In species that produce resting cysts, encystment takes place after the planozygote loses motility. The cell contents decrease and development of a thick wall occurs within the outer membrane of the planozygote (Figueroa & Bravo 2005a). There are several different descriptions in the literature about the transformation from planozygote to hypnozygote and the formation of resting cysts. The main feature of encystment is the replacement of the theca by a thick wall and rapid increase of starch granules and the reduction in size or disappearance of the cytoplasm structures (Bibby & Dodge 1972). Kokinos and Anderson (1995) described encystment of *Lingulodinium polyedrum* as beginning with several simultaneous events: (1) the cell (planozygote) loses motility and comes to rest, (2) the flagella are released from from the cingular grooves, (3) the external membrane swells and bubble-like protrusions form on the surface of the cell, (4) the theca separates along one or more plate sutures, (5) a single layer of the globules become visible in the space between the theca and the cytoplasm, and (6) the cyst processes expand into the intervening space.

Sarjeant (1974) described the process of a planozygote changing into a resting cyst. As a wall develops around the external planozygote, well-separated granules appear and a hyaline interstice develops between the wall and the protoplast surface. The granules then develop into spines and a thick cellulosic surrounding wall (exospore). The initial wall (endospore), which is thin and ruptures away to one side, is absorbed and the interstice becomes a vacuolate mass to form a protuberance on the same side.

The contractional growth hypothesis for the cyst formation described by Sarjeant was not supported by Dale (1983). He suggested that during encystment of spiny cysts (e.g. *Gonyaulax grindleyi* Reinecke, *G. polyedra, Gonyaulax spinifera* Kofoid), newly forming cysts with immature process completely occupy the thecal lumen. Cysts with relatively long process do not form by contraction of the cyst body, as the process of growth helps to break up the theca by an overall development of the cyst.

Furthermore, Gao *et al.* (1989) have documented development of a hypnozygote for *Scrippsiella* sp. by using electron microscopy. The planozygote has two longitudinal flagella and one transverse flagellum and the cytoplasm contain starch granules, a few lipid droplets, lamellae, Golgi body, mitochondria and nucleus. The planozygote may remain motile for 2-3 days, as the planozygote develops organelles in the cytoplasm which decrease the size and number that are necessary for encystment. In the early stage of encystment, the cell is surrounded by three membranes. The cyst wall is then raised between the middle and inner membrane and the muciofibrous spines forms between the outer and middle membrane at early stage of encystment. Middle aged cysts are surrounded by an outer membrane and muciofibrous material, some fibrous material is lifted to form projections that are later calcified as encystment continues. Clearly the process differs significantly between species.

There are also several reports on time taken for planozygotes to encyst, usually 1-2 weeks (Anderson *et al.* 1985; Blackburn *et al.* 1989), sometimes as long as 70 days or as short as 3-4 days (Figueroa *et al.* 2005).

4.1.2. Cyst Formation in the Laboratory

Encystment is a complex phenomenon whereby the physiological responses of the vegetative population are changed by several environmental factors. The first detailed study of encystment of dinoflagellates in the laboratory investigated the freshwater species, *Ceratium hirundinella* Muller Bergh (Huber & Nipkow 1922). In the last few decades, the majority of work on encystment and cyst forming has been carried out in laboratory culture experiments (e.g. Anderson *et al.* 1983; Ellegaard *et al.* 1998; Uchida 2001).

Some common factors such as nutrient depletion, light intensity and temperature induce cyst formation in the culture. However, sexuality and subsequent encystment can occur in culture without obvious stimulus (e.g. *Scrippsiella trochoidea* Braarud; Blanco 1995), but usually for most species, encystment must be induced by some change in culture. Factors that induce encystment appear to be a species specific characteristic (Table 2).

For example, cysts of *Alexandrium tamarense* (Lebour) Balech are produced when nutrients are limited (Anderson *et al.* 1984; Kremp & Heiskanen, 1999). Blanco (1995) has shown that mainly phosphorous or nitrogen deficiency are the most important factors inducing encystment in *Scrippsiella trochoidea*, *Ensiculifera* sp., *Alexandrium lusitanicum* Balech and *Lingulodinium polyedrum* (Stein) Dodge. Blanco (1995) also suggested that, among other deficiencies (Fe, Cu, vitamin B_{12}), iron deficiency affected only *A. lusitanicum*.

Another factor that may induce encystment is temperature. Surveys on dinoflagellate cyst formation have illustrated that encystment usually happens at specific temperatures. Anderson *et al.* (1984) and Sgrosso *et al.* (2001) suggest that cyst formation in *Scrippsiella trochoidea* var. *aciculifera* Montresor and *Scrippsiella rotunda* Lewis is controlled by a complex interaction of at least 3 factors: temperature, day length, and nutrient concentration. Temperature and day length were the main factors, while nutrient concentration modified the intensity of cyst formation. High encystment occurred only at shortest day lengths.

Some other factors have also been reported to explain the encystment: interaction with cyst-inducing bacteria (Adachi *et al.* 1999), biological condition such as defence from predators (Rengefors & Anderson 1998), and *S. trochoidea* reduced the cyst production efficiency of *L. polyedrum* in unialgal cultures (Blanco 1995).

Table 2: Summary of investigated factors that induce encystment in different dinoflagellate species

Species	Encystment	References
Alexandrium catenella	Nutrient limitation	Figueroa *et al.* (2005)
Alexandrium minutum	Nutrient limitation	Garces *et al.* (2004)
Alexandrium tamarense	Nutrient limitation	Anderson *et al.* (1984); Ichimi *et al.* (2001)
Alexandrium lusitanicim	Iron, Phosphorous, nitrogen deficiency	Blanco (1995)
Calciodinellum operosum	Day length,	Sgrosso *et al.* (2001)
Gymnodinium sp.	-N & -P media	Present study
Gymnodinium catenatum	-N & -P media, nutrient limitation	Band-Schmidt *et al.*(2004) Blackburn *et al.* (1989)
Lingulodinium polyedrum	N & P deficiency	Blanco (1995)
Scrippsiella trochoidea	N & P deficiency	Blanco (1995)
S. irregularis	N & P deficiency	Present study
Scrippsiella cf. *lachrymosa*	Nutrient depletion	Olli & Anderson (2002)
S. trochoidea var. *aciculifera*	Day length (8L:16D or16L:8D)), and low temperature (15°C)	Sgrosso *et al.* (2001)
S. rotunda	Day length (8L:16D or 16L:8D) and temperature (25°C)	Sgrosso *et al.* (2001)

4.1.3. Encystment in the Natural Environment

Encystment has been investigated in natural environments by many authors (e.g. Anderson *et al.* 1983; Montresor 1998; Kremp & Heiskanen 1999). In nature, it has been suggested that cyst formation is generally seasonal and dependent on the abundance of vegetative cells in the water column. For sexual reproduction and encystment to happen, a threshold concentration of gametes may be required to produce the zygote and then form the cyst (Wyatt & Jenkinson 1997). Species that reach their maximum abundance or bloom in spring, form cysts in late spring early summer (e.g. *Protoperidinium oblongum* (Aurivillius) Parke & Dodge; Wall & Dale 1968) and species with maximum abundance in summer form cysts in late summer/autumn (e.g. *Lingulodinium polyedrum*; Dale 1983).

Dale (1983) suggested that species with two annual peaks of maximum abundance, usually in spring and autumn, formed cysts following each peak (e.g. *A. tamarense*). Some species, such as *S. trochoidea,* have periodic abundance and their cyst formation expands over spring, summer and autumn (Dale 1983). Another example of this is *S. rotunda* which has high encystment in September and October, when day length is short and also in May and June, when day length is longest (Montresor *et al.* 1998).

Many other potential factors have been investigated in cyst formation and encystment. In culture experiments nutrient depletion is a major factor in cyst formation. However, Anderson *et al.* (1983), Kremp & Heiskanen (1999) and Godhe *et al.* (2001) have suggested that there is no evidence of nutrient limitation inducing encystment in nature. The other factors that may influence cyst inducment is endogenous processes or biological factors, such as pheromone-like substances (Pfiester 1975).

4.1.4. Encystment in Tropical and Sub-tropical Areas

Information about factors inducing encystment has mainly been gathered from middle and higher latitudes species and information about tropical and sub-tropical areas are few. The existing information in these areas related to water temperature, salinity and nutrient in general and to monsoon currents in particular. In tropical waters, cyst formation is less influenced by plankton abundance and plankton blooms (Dale 1983). In Somalia, the highest cyst formation of calcareous dinoflagellate was observed during the late southwest monsoon (May) with high nutrient concentration and increased stratification (Wendler *et al.* 2002). In north-west Africa, cysts of *Protoperidinium americanum* (Gran & Braarud) Balech are produced with high surface water nutrients and relatively low water temperature (Susek *et al.* 2005). Pena-Manjarrez *et al.* (2005) showed that resting cysts are produced in Todos Santos Bay (subtropical area, Mexico) when a combination of three factors fell below certain levels; temperature (temp<16°C), dissolved inorganic nitrogen (<0.5µM nitrite/nitrates) and irradiance value (<150µmol quanta $m^{-2}s^{-1}$). *G. catenatum* from

Bahia Concepcion Gulf of California (Mexico) produced resting cysts in nutrient depleted media (Band-Schmidt *et al.* 2004).

In Somalia and Pakistan, dinoflagellate cyst production and distribution in surface sediment are affected by surface circulation in the Arabian Sea which is driven by semi-annual reversing monsoon winds (Wendler *et al.* 2002). *Polykrikos kofoidii* Chatton and *Spiniferites ramosus* (Rossignol) cysts relative abundance depends on the water temperature and salinity in upper water column of the Arabian Sea and these factors are also strongly influenced by seasonal forcing of the monsoon currents (Zonneveld 1997).

4.2. Dormancy and Quiescence

Following encystment, cysts remain dormant on the sea floor before they are capable of germinating. Dormant cysts cannot germinate, even under favourable conditions, and may be inhibited by an active endogenous annual clock which regulates germination to a particular time of the year (Anderson *et al.* 1995). Once the mandatory dormancy period is completed, the cyst can germinate under optimum conditions or remain in sediment for several years if environmental conditions are not suitable. This period is termed quiescence. The quiescence has been referred to as the suspension of growth by unfavourable environmental conditions (Matsuoka & Fukuyo 1995). Thus quiescent cysts are capable of germinating under favourable environmental conditions.

The length of cyst dormancy varies between species (from 12 hours to 1 year (Pfiester & Anderson 1987; Perez *et al.* 1998), and is species specific (Table 3). Dormancy length can also vary for a single species. Several investigations show that temperature is a major factor that can affect the length of dormancy. Pfiester (1975) reported that cyst of *P. cinctum,* in culture held at 20°C, germinated after 7-8 weeks but held at 4°C did not germinate for at least 5 months. In contrast, dormancy of *Alexandrium pseudogonyaulax* Biecheler that have been exposed to cold dark conditions for 1.5-2 months showed a shortened maturation time from 90 days to 75 days (Montresor & Marino 1996). Dormancy period has generally been considered a

time for physiological maturation (Pfiester & Anderson 1987) and has also been termed as maturation period.

Table 3: Known dormancy periods of different dinoflagellate species

Species	Dormancy	References
Alexandrium monilatum	14-28 days	Walker & Steidinger (1979)
A. pseudogonyaulax	189 days (20°C)	Montresor & Marino (1996)
A. tamarensis	5 months	Anderson (1980)
A. taylori	15 days	Giaccobe & Yang (1999)
Ceratium hirundinella	4.5 months	Rengefors & Anderson (1998)
Gymnodinium catenatum	14 days	Blackburn *et al.* (1989)
G. nolleri	26-40 days	Figueroa & Bravo (2005b)
Gonyaulax digitale	3 months	Dale (1983)
Peridinium gatuense (Freshwater pond)	12 hours	Pfiester (1975)
P. cinctum (FP)	49-56 days	Pfiester (1975)
P. willei (FP)	46-56 days	Pfiester (1975)
Scrippsiella trochoidea	25 days	Binder & Anderson (1987)
Scrippsiella trifida	One week	Head *et al.* (2006)
Scrippsiella rotunda	17-24 weeks	Nuzzo & Montresor (1999)
S. hangoei	6 months	Kremp &Anderson (2000)
S. cf. *lachrymosa*	60 days	Olli & Anderson (2002)

The biochemical activity of cysts during the dormancy period is poorly known. Dale (1983) suggested that metabolic activity of the cysts significantly reduced so they can remain viable for many years under unfavourable conditions. Cell contents of autotrophic species becomes darker and may represent storage of photosynthetic pigments (Yentsch *et al.* 1980); many non-photosynthetic cysts (e.g. *Protoperidinum* spp.) cell contents remain pale throughout this period and some show no obvious change before encystment. In addition, Binder and Anderson (1990) suggested that cysts of *Scrippsiella trochoidea* show reduced respiration rates (10% of rate of motile cell). Rengefors *et al.* (1996) have also documented that dinoflagellate cysts are able to take up phosphorous (P) during dormancy and quiescence period. They also suggest that *Scrippsiella* cysts may be capable of nitrogen (N) uptake.

4.3. Excystment and Quiescence

The process of excystment has been described by Dale (1983). Prior to excystment, the cell contents frequently pull away from the cyst wall and the cingulum and other major features of body shape may begin to form. The archeopyle then opens and the protoplast flows through the archeopyle by an amoeboid-like movement. The longitudinal flagellum is formed just before, or few minutes after germination, then the transverse flagellum expands. After excystment, the theca is formed for armoured dinoflagellates. Generally, excystment has been documented as a biologically challenging process involving a change from one physiological condition to another (Pfiester & Anderson 1987).

The factors inducing germination of quiescence cysts are known for some species (Table 4). Excystment can be inhibited by external environmental conditions such as temperature (Anderson *et al.* 1987). However, spontaneous germination of cysts without any change in temperature has also been documented (Binder & Anderson 1987). Many authors have suggested (e.g. Wall & Dale 1968; Anderson & Morel 1979) that temperature is a major stimulation for excystment of temperate species in the natural environment. *C. hirudinella* germinates in less than 36 hrs at an optimum temperature of 21-22°C, but takes a longer time to germinate at both higher and lower temperatures. A "temperature window" is one within which the quiescent cysts can excyst, time permitting, but outside of which germination is inhibited (Dale 1983). Therefore, once dormancy is completed at a temperature within the window, excystment will arise as a natural result of the end of dormancy without any further stimulation. Temperature is therefore an important factor in the dynamic of dormancy quiescence and excystment of temperate species.

Table 4: Summary of investigated factors that effect germination of different dinoflagellate species

Species	Excystment	References
Alexandrium tamarense	Anoxia (I), darkness (S), salinity < 30 ‰ in summer monsoon (S)	Anderson *et al.* (1987); Anderson (1980); Perez *et al.* (1998); Kim *et al.* (2002)
Gonyaulax verior	Anoxia (I), darkness (S)	Anderson *et al.* (1987)
G. rugosum	Anoxia (I)	Anderson *et al.* (1987)
Lingulodinium polyedrum	Anoxia & darkness (I)	Anderson *et al.* (1987)
Scrippsiella sp.	Anoxia (I), darkness (S)	Anderson *et al.* 1987
Scrippsiella throchoidea	Nutrient deficiency (S), lower than 14°C (S), 22°C -25°C(H),	Binder & Anderson (1987); McQuoid (2005)
Scrippsiella hangoei	Turbulent water, darkness (S) Temperature 0-9°C (H), anoxia(I), high sulphide (S)	Kremp (2001); Kremp & Anderson (2000);
Peridiniella catenata	Turbulent water	Kremp (2001)
Gymnodinium catenatum	Darkness (S), 22-28°C(H), >30°C or <11°C (I), high temperature	Bravo & Anderson (1994)
Gymnodinium nolleri	15‰ salinity (I) but germinate at 25‰ & 35‰,	McQuoid (2005)

Germination, inhibited (I), High rate (H), Slow rate (S)

Germination may also be affected by various environmental factors such as light, chemical composition of the water, anoxia, biological factors such as endogenous rhythms and deposit feeder ingestion. Most past studies (e.g. Dale 1983; Anderson *et al.* 1987) have suggested that light has less effect on the germination. For example Anderson *et al.* (1987) suggested that darkness delayed excystment of *A. tamarense*, but did not prevent it. However, a short exposure to low light during handling can not be ignored. Kremp (2000) illustrated that germination of *Scrippsiella hangoei* (Schiller) Larsen cysts increased considerably in the light, but incubation in darkness did not prevent excystment. She suggested that germination does not happen in natural sediment without light stimulation.

Nutrient concentrations also do not significantly affect cyst germination of either *G. catenatum* (Bravo & Anderson 1994) or *A. tamarense* (Anderson & Wall 1978). However, Binder and Anderson (1987) found a noticeable delay in cyst germination of *S. trochoidea* when kept in medium without nutrients. An *et al.* (1992) also showed that decreasing salinity and increasing nutrient concentration were linked with raised germination rate of *Scrippsiella* and *Gonyaulax* species.

The excystment of most dinoflagellate species appear inhibited under anoxic conditions. Anoxia causes complete inhibition of germination of *A. tamarense, Gonyaulax verior* Sournia , *Gonyaulax rugosum* Wailes, *L. polyedrum,* and *Scrippsiella* sp. (Anderson *et al.* 1987) and *Scrippsiella hangoei* (Kremp & Anderson 2000). However, some species can germinate under very low oxygen concentrations (e.g. *Ceratium hirundinella*, Rengefors & Anderson 1998). It has also been demonstrated that several dinoflagellate cyst species can be successfully germinated after long periods of burial in anoxic sediment usually associated with high sulphide concentration (Keafer *et al.* 1992).

Resuspension and water turbulence is another environmental parameter that may affect cyst germination. Small scale turbulence reduced excystment of *S. hangoei* but did not affect the germination of *Peridiniella catenata* (Levender) Balech so, it is species specific (Kremp 2001). Salinity is the other factor that may influence cyst germination. Kim *et al.* (2002) have suggested that germination of *Alexandrium tamarense* is lowest during summer monsoon in tropical areas, when sea surface salinity drops below 30%. Germination of *Alexandrium minutum* Halim is slow in salinity lower than 30%; however, it shows adaptation to lower salinities in the range 14-26% (Kim *et al.* 2002). In addition, McQuoid (2005) showed that *Gymnodinium nolleri* Ellegaard & Moestrup did not germinate at 15% salinity but successfully germinated at 25% and 35% salinity.

Deposit feeder ingestion may affect on survival and excystment of the cysts. Cysts can easily be buried in sediment by bioturbation, therefore can not germinate due to lack of light and oxygen. However, Ichimi and Montani (2001) found that ingestion by deposit feeders such as polychaetes in deeper layers transfers cysts to the surface and may help in survival of viable cysts. Cysts of *Alexandrium catenella* (Whedon &

Kofoid) Balech, *Gymnodinium* sp., *Scrippsiella trochoidea* were germinated after isolation from the fecal pellets of deposit feeders in surface sediments.

Some general ecological implications of the laboratory results can be considered in the context of natural populations. Species have different mandatory dormancy periods, light requirements, and variable sinking rates (Anderson *et al.* 1985). Anderson *et al.* (1987) reported that species with short mandatory period, minimal light requirement and low sinking rates (e.g. *S. trochoidea* and *G. rugosum*) may cycle rapidly between a resting state and a motile stage. Therefore, these species can maximise their contribution to the bloom initiation phase. Species with high sinking rates and long dormancy periods and a need for light (e.g. *A. tamarense*) fall to the bottom before germination can occur. Only those cysts that receive sufficient light and oxygen can germinate, therefore their contribution to the onset of a bloom might be smaller. In marine environments, many cysts will be exposed to anaerobic conditions or bioturbation and burial in the sediment. These cysts, although they can survive without oxygen, can not germinate and contribute to future populations.

In summary, germination of dinoflagellate cysts is a complex phenomenon that is species specific and regulated by several environmental factors. Temperature, nutrient concentration, light regime, anoxia, turbulence and maturation time of the cysts are all important factors that may affect germination.

5. Biological Importance of Resting Cysts

Several roles and functions of cysts have been hypothesised by previous studies (e.g. Dale 1983; Anderson *et al.* 1983; Bolch *et al.* 1991; Hallegraeff 1995b; Kremp 2000; Dale & Montresor 2001). Based on these studies, the main biological functions of resting cysts can fall into the following categories:

1. ***Cysts act as "seed population" or "seed beds" for initiation and decline blooms:*** Cysts formed through the sexual life cycle can germinate and re-establish the motile population in the water column to initiate a bloom. The potential importance of resting cysts and their "seed bank" role in the initiation of bloom has been

highlighted in several studies (e.g. Anderson & Wall 1978; Anderson *et al.* 1983). The germination of *Scrippsiella hangoei* can provide an inoculum of motile cell in the water column for bloom initiation in the northern Baltic Sea (Kremp & Anderson 2000). In addition, high abundance of *Lingulodinium polyedrum* resting cysts were found in Todos Santos Bay (Mexico, subtropical), and the area is considered as a seed source for blooms of this species (Pena-Manjarrez *et al.* 2005). However, cysts of *Gymnodinium catenatum* are considered to play a minimal role in seasonal bloom dynamics in southern Tasmanian waters (Hallegraeff *et al.* 1995).

Cysts may also have a role in bloom decline by decreasing cell numbers through the sexual fusion of two gametes to produce a zygote. Blooms of *Lingulodinium polyedrum* declined in the Setubal Bay of Portugal during cyst formation (Amorim *et al.* 2001). There is a direct relationship between high occurrence of motile cells of *A. minutum* (in April) and increasing of cysts (in May) in Kuwait (Husain & Lewis 2004), which suggest that cyst formation has contributed to the decline of blooms.

2. ***Cysts can be a vector for dispersal of dinoflagellates:*** Cysts and planktonic cells can be transported to other areas by natural coastal and oceanic currents. However, cysts are resistant to environmental stress and may be a more successful dispersal vector. Dinoflagellate cysts behave as silt-like particles in the water column and can easily be translocated by oceanic currents, such as monsoon currents (Anderson *et al.* 1985). Zonneveld (1997) suggested that cysts of *Pyrodinium bahamense* Plate (= *Polysphaeridium zohari* (Rossignol) Bujak *et al.*) are transported to the Arabian Sea from Red Sea and Gulf of Oman by the SW monsoon current.

Cysts can also be translocated by human activity (e.g. ballast water from ships or via transport of shellfish stocks), resulting in the introduction of HAB species into the new areas. Chain-forming *G. catenatum* was first found in southern Tasmanian waters in 1980, since then it has produced blooms annually (Hallegraeff *et al.* 1988). Cysts of *G. catenatum* have been found in the ballast water of ships entering Australian ports from Japan and Korea (Hallegraeff & Bolch 1991, 1992). *Heterosigma akashiwo* Hada (Raphidophyte) is a bloom-forming species and its blooms associated with massive fish mortalities. Dominic and Connell (2000) has suggested that cysts have been spread via ship's ballast from Japan to California,

seeding two blooms in 1998. *Heterocapsa circularisquama* first appeared in Japan in 1988, and since then massive blooms have caused damage to shellfish aquaculture (Matsuyama 1999). It has been suggested that this species was introduced from tropical to subtropical waters to Japan via transfer of stock of juvenile oysters and subsequently spread within Japanese water by movement of oyster spat (Matsuyama 1999).

3. ***Genetic recombination:*** Resting cysts are a zygotes resulting from sexual fusion of gametes and presumably undergo sexual recombination. Genetic heterogeneity is documented within dinoflagellates (Walsh *et al.* 1998; Bolch *et al.* 1999) and there are many examples of how variation in ecophysiological characteristics such as temperature, salinity and tolerance reflects genetic diversity (Scholin & Anderson 1993; Scholin *et al.* 1994). Intraspecific variability influences the physiological response (such as growth rate, encystment and excystment) of a species to different environmental conditions. In terms of population dynamics, most variations might have adaptive significance. Therefore, it is important to know which intraspecific variability is related to genetic differences that regulated by environmental factors.

4. ***Protection from sub-optimal conditions:*** Cysts have a protective role for the population as they can survive when the conditions are not suitable for motile cells. *Alexandrium tamarense* encysted in the late spring as water temperature increased, excysted several months later when water temperature decreased (Anderson 1980). Culture experiments also show that cysts survive under adverse conditions. Anderson *et al.* (1987) showed that *Lingulodinium polyedrum* cysts remained viable in culture under anaerobic dark conditions for several months. Large numbers of living cysts accumulate below the surface marine sediment because of inhibitory effect of darkness, temperature, and ability of anoxia to germinate in the optimal conditions (Anderson *et al.* 1983). Bravo and Anderson (1994) found an optimal temperature for excystment of *Gymnodinium catenatum* ranging between 17-29°C and outside this range germination was reduced and cysts remained quiescent.

6. Resting Cysts, Harmful Algal Blooms and Shellfish Toxicity Monitoring

In recent years knowledge of phytoplankton cysts has significantly increased, and utilised as a useful tool to aid phytoplankton monitoring in shellfish areas. A better understanding of the factors regulating the dynamics of (Harmful Algal Bloom) HAB populations including environmental, human influences and identification of all life stages at a species-specific level is useful for monitoring and prediction of HABs. The ultimate goal of HAB monitoring is to predict species-specific events in the specific areas. Successful prediction of the location, timing and magnitude of harmful algal blooms could mitigate their impact on the aquaculture industry (Hallegraeff *et al.* 1995).

Several HAB species produce resting cysts and some have the capability to fossilise (e.g. *Pyrodinium bahamense* (Böhm) Steidinger, and *Gymnodinium catenatum*). These cysts provide evidence of the global spreading or recession of HABs species. *Pyrodinium bahamense* is presently restricted to coastal waters of the tropics and subtropics, but fossil records, dating back to the Pleistocene, show that the species range reached to higher latitudes in both hemispheres in the past (Matsuoka *et al.* 1989).

Pyrodinium bahamense blooms are a serious public health and economic problem in tropical coastal areas. The first PSP outbreak in Philippines in 1983 caused a total prohibition on the harvest and sale of all shellfish for 8 months (Hallegraeff 1993). A bloom of this species also resulted in paralytic shellfish poisoning in Papua New Guinea that caused fatal food poisoning of three children (Hallegraeff 1993).

Known harmful algal species that produce resting cysts are summarised in Table 5. Cysts may also be a source of toxin for benthic shellfish. Some benthic shellfish have very limited opportunity of feeding on planktonic cells. For these species, cysts of toxic species that accumulate on the surface of the sediments could be a toxin source. *Alexandrium* resting cysts are known to be more toxic than vegetative cells (Oshima *et al.* 1992). The cysts of *Alexandrium tamarense* have been found in the gut contents of sea scallops and surfclams of the Gulf of Maine (Cembella *et al.* 1994).

Table 5: Known HAB or toxic species (Dinoflagellate and Raphidophytes) with a resting cyst

Species	Harmful effect	References
Alexandrium catenella	PSP	Adachi *et al.* (1999); Hallegraeff (1998); Vila *et al.* (2001)
Alexandrium minutum	PSP	Erard-Le Denn *et al.* (2000)
Alexandrium monilatum	Ichthyotoxic	Walker & Steidinger (1979)
Alexandrium ostenfeldii	Spirolides (SST)	Jensen & Moestrup (1997)
Alexandrium tamarense	PSP	Wyatt & Jenkinson (1997)
Alexandrium taylori	Water discolouration	Garces *et al.* (1998)
Gymnodinium catenatum	PSP	Bravo & Anderson (1994); Blackburn *et al.* (2001)
Pfiesteria piscicida	Neurotoxic, Ichthyotoxic	Burkholder *et al.* (2001) Glasgow *et al.* (2001)
Pfiesteria shumwayae	Neurotoxic, Ichthyotoxic	Glasgow *et al.* (2001)
Protoceratium reticulatum	Yessotoxin	Faust (1990), Faust (1997)
Prorocentrum lima	DSP	Pan *et al.* (1999)
Pyrodinium bahamense	PSP	Matsuoka *et al.*(1989)
Lingulodinium polyedrum	Yessotoxin	Paz *et a*l. (2004)
Chattonella antiqua	Ichthyotoxic	Haque & Onoue (2002)
Chattonella marina	Ichthyotoxic	Haque & Onoue (2002)
Fibrocapsa japonica	Ichthyotoxic, Neurotoxins	Band-Schmidt *et al.* (2004)
Heterosigma akashiwo	Ichthyotoxic	Twiner & Trick (2000)
Scrippsiella trochoidea	Anoxia	Hallegraeff (2002)
Ostreopsis siamensis	Palytoxin	Rhodes *et al.* (2000)

Blooms of toxic species that cause shellfish toxicity or fish killing are usually short—planktonic cells accumulate in thin layers within water column over limited time intervals and the causative species may be collected and identified. However, cysts of toxic species remain in the sediment after a bloom and can be separated,

germinated and examined for toxicity. This way, resting cysts can be used as a long-term integrated record of toxic species in an area.

In recent years the aquaculture industry has been developed as an economically important activity in tropical and subtropical coastal areas. Despite this fact, monitoring programs for detection of known harmful species and toxin levels in shellfish and fish are lacking or very limited in many tropical and sub-tropical areas (including Iran). Performing regular cyst surveys can help in detecting areas that are free of potentially harmful species and favourable for aquaculture. Or areas that are suitable for HABs' species growth, and thus risky areas for intensive aquaculture, can be identified. Furthermore, cyst surveys can provide a baseline for monitoring of HAB spreading events, the introduction of new species, human-assisted dispersal of HAB species and development of blooms (Garces *et al.* 2002, 2004).

7. Research Aims

Harmful algal blooms that affect marine ecosystem and fisheries are becoming a serious and increasing economic problem in Iran. The south coast of Iran has a growing shrimp farming area and has potential as a shellfish aquaculture area. Mapping cyst beds in surface sediments will provide useful information to consider potential risk of HABs for new aquaculture activities. As the presence of dinoflagellate cysts of toxic species in an area, indicates the potential danger for future blooms, it is essential to document the resting cysts flora of potential aquaculture areas in south-eastern Iran.

This work will be the first research on dinoflagellate cysts in Iranian coastal waters. The main objective is to identify dinoflagellate cysts and other potentially harmful resting stages along southeast coast of Iran.

The specific aims are:

1. To describe the recent dinoflagellate cyst and resting stages flora of other HAB flora of the south coast of Iran.

2. Establish the identity of unknown resting stages by using germination experiments.
3. To combine morphological, molecular and toxicological approaches to aid the characterisation and description of new dinoflagellate and HAB species discovered during the study.
4. Examine the molecular phylogeny of any new species using DNA sequences of the large-subunit ribosomal RNA (rDNA) gene and rDNA-intergenic spacer sequences.
5. Relate the relative abundance and distribution of the dinoflagellate cyst flora to the known monsoonal weather patterns and oceanographic features of the Oman Sea and south-east coast of Iran.

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CHAPTER 2

Dinoflagellate Cysts from Recent Marine Sediment of the Southeast Coast of Iran (Northeast of the Gulf of Oman)

1. Introduction

The study of marine dinoflagellate cysts is an important area of research for both palaeontologists and biologists (Dale 1983). Many marine dinoflagellate species have a resting cyst as part of their life history, and more than 260 species of dinoflagellate are known to produce a resting cyst (Head 1996). Resting cysts formed during blooms settle to the sediment and can survive for a long time. The cysts in sediments function as seeds for dinoflagellate populations in the water column (Bolch & Hallegraeff 1990). In appropriate environmental conditions, resting cysts germinate to re-establish the species in the water column. Dinoflagellate cysts in an area can represent species that have not been found previously in the plankton motile stage. Uncommon or short lived species can be easily missed during plankton surveys, or in some areas can be difficult to sample in particular seasonal conditions, therefore sediment sampling can be a useful addition to plankton surveys (Dale 1983). Dinoflagellate cysts also provide a robust means of dispersal. Hallegraeff and Bolch (1991) demonstrated the potential for new areas by transportation of cysts in ship's ballast water. Analysis of resting cysts also provides a useful means of surveying areas for toxic or harmful dinoflagellates that have bloomed in an area over a number of years (Matsuoka 1999).

A number of qualitative and quantitative surveys of dinoflagellate cysts from recent sediments from the northeast of Indian Ocean (Persian Gulf, Arabian Sea, Oman Sea) have been carried out (Bradford 1975; Bradford & Wall 1984; Zonneveld 1997, Zonneveld *et al.* 2001; Zonneveld & Jurkschat 1999). Some other palaeontological studies have also been carried out in the Arabian Sea (Oman Sea), for example, as part of the Netherlands Indian Ocean Programme (NIOP, 1992-1993), by Wendler *et al.* (2002). While most studies have been palaeontological studies, Husain and Lewis (2004) surveyed living marine dinoflagellate cysts in Kuwait sediments.

In recent years, Harmful Algal Blooms (HABs) have become an increasingly serious problem along the southern coast of Iran bordering the Gulf of Oman due to their affect on marine ecosystems, fisheries and aquaculture. The region has several existing shrimp farms and is targeted as a potential area for expansion of shellfish aquaculture. Red tides also occur in southeast coast of Iran (Gulf of Oman) every year after southwest monsoon (October-November), these are sometimes associated with high mortality of fish and other marine organisms (Shakoory 2004).

Dinoflagellate cysts have never been surveyed in the Iranian waters of the Gulf of Oman. Due to the potential expansion of aquaculture, it is important to identify potentially toxic dinoflagellates in the area. This research aims to examine the species composition and abundance of recent marine dinoflagellate cysts from these potential aquaculture areas along the southeast coast of Iran, to provide information on potentially toxic or harmful dinoflagellates that may affect the development of Iranian shellfish culture industry.

2. Material and Methods

2.1. Study Area

The southeast coast of Iran is located in the tropical climatic zone in the northern part of the Gulf of Oman, in the northwest of Arabian Sea, and in the northwest of Indian Ocean. The Gulf of Oman is connected to the Persian Gulf from the west by the Straits of Hormuz. The oceanography of the Arabian Sea is strongly controlled by

the semi-annual reversal of the monsoon season; the southwest (SW) monsoon occurs during summer (June-August) and the northeast (NE) monsoon occurs during winter (September-May) (Zonneveld & Jurkschat 1999).

Three sediment samples were collected by Ekman grab (with a sampling area of 0.225m^3) from each of three locations along the southeast coast of Iran (from 25° 17'N 60° 24'E to 25° 11'N 61° 34'E) in March 2004. Fig. 1 shows the sediment sampling locations. The date of sampling, water salinity, temperature, depth, and sediment type of each station are listed in Table 1. Bahoo-Kalat is in the eastern part of the south coasts of Iran. This estuary from its middle to its end is surrounded by mangrove trees. Its entrance has sandy-silt sediment without mangrove trees. There are several active shrimp farms in this area. Sediment samples were collected from the entrance of the estuary where the water supplies the shrimp farms. Pasabandar is a coastal area between Chabahar and Bahoo-Kalat. It is characterised by common occurrences of HAB events (Khodami 2005 pers.com., Iranian Fisheries Research Institute-Chabahar Centre). Chabahar Bay is located in western part of our study area, almost in the middle of the southeast Iran coast near the town of Chabahar. This bay is the main fishing ground for lobsters in the region (Nikouyan *et al.* 1998).

Site selection was based on sediment characteristics, occurrence of past dinoflagellate blooms, and proximity to aquaculture areas. The top sediment layer of samples was bottled and stored in the dark at 20°C in icebox, prior to air-freight transport to the University or Tasmania, School of Aquaculture laboratories for sample processing. Sample transit was 5 days from origin in Iran to destination.

All samples were imported to Australia under valid Australian Quarantine Inspection Service (AQIS) permits for import of biological materials (issued to the School of Aquaculture, University of Tasmania) and transferred "under bond" via domestic couriers to the Quarantine Approved Premises at the School of Aquaculture. All research activities (i.e. LM, SEM, Cyst incubation, PCR, DNA sequencing) were carried out at the University of Tasmania in the School of Aquaculture (Launceston), School of Plant Science (Hobart), and the Central Science Laboratory (Hobart). In addition, some SEM examination was carried out at the Australian Antarctic Division in Kingston, Hobart, Tasmania.

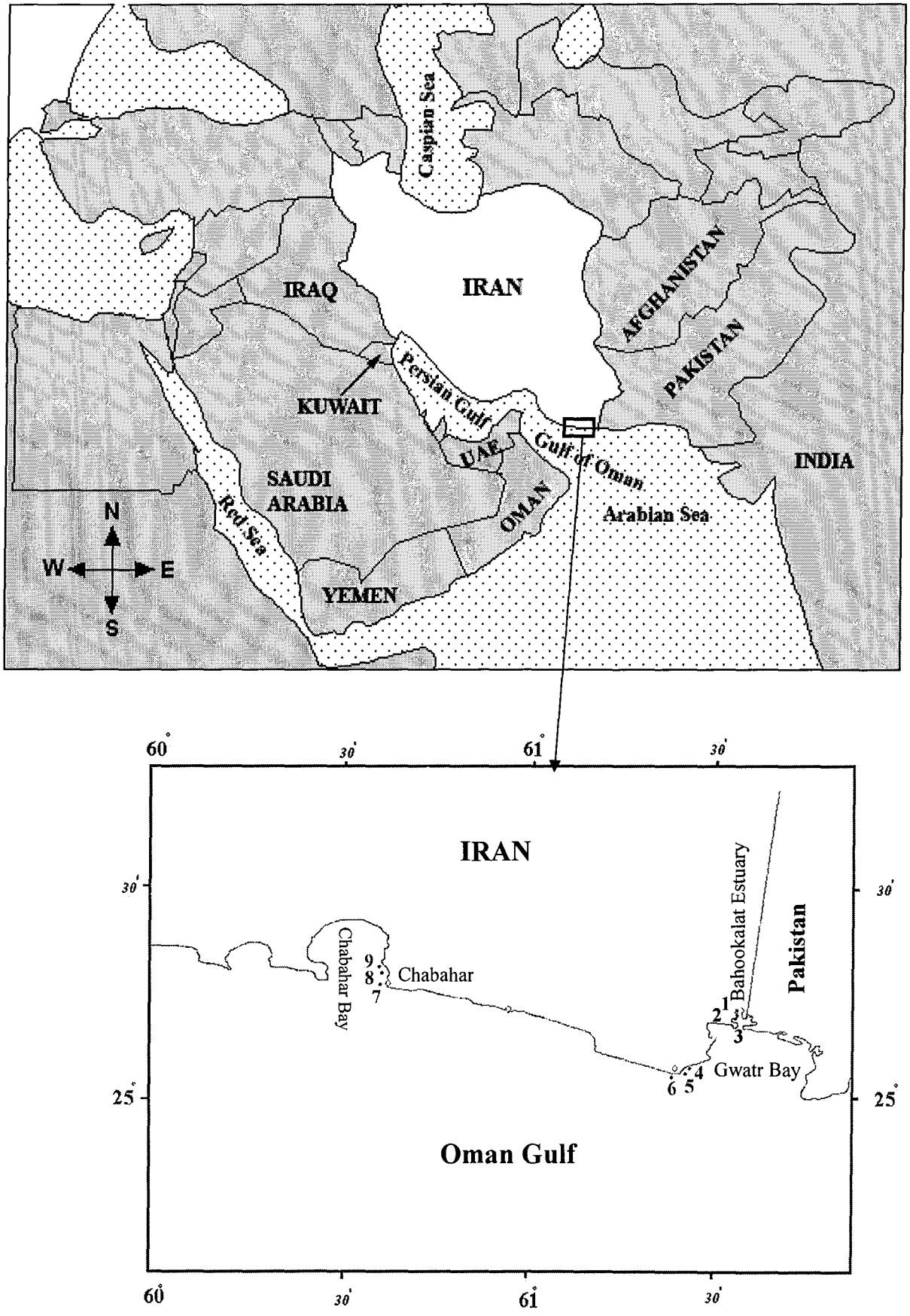


Fig. 1. Map of Study Area

Table 1: Description of sampling stations

Sites	Locations	Depth (m)	Salinity (ppt)	Temp (°C)	Sediment Type	Sampling Date
1	Bahoo-Kalat	1.7	39.1	21.9	Sand –silt	16.03.2004
2	Bahoo-Kalat	2.5	38.5	22.0	Sand –silt	16.03.2004
3	Bahoo-Kalat	1.5	37.9	22.2	Sand –silt	16.03.2004
4	Pasabandar	2.0	37.5	25.3	Black mud	17.03.2004
5	Pasabandar	3.0	37.5	25.4	Black mud	17.03.2004
6	Pasabandar	4.5	37.5	25.3	Grey mud	17.03.2004
7	Chabahar Bay	3.0	37.5	25.1	Clay-silt	18.03.2004
8	Chabahar Bay	4.0	37.5	25.0	Clay-silt	18.03.2004
9	Chabahar Bay	3.0	37.5	24.9	Clay-silt	18.03.2004

2.2. Sediment Processing

Approximately 1-3g wet weight of sediment was taken from each sample and mixed with filtered seawater (FSW) to obtain a watery slurry. The sediment suspensions were sonicated for 2 minutes by Microson Ultrasonic cell disruptor, to separate detritus particles. Each sample was then washed through a 125µm sieve and collected on a 20µm mesh sieve using filtered seawater (Bolch & Hallegraeff 1990). The resting cysts were then concentrated by density centrifugation through sodium polytungstate at a specific gravity of 1.3 and 1.6 g cm^{-3}, as described by Bolch (1997). Sub-samples of concentrated cysts were examined on glass slides for identification of dinoflagellate cysts.

2.3. Quantitative Analyses of Cysts

Known wet weights of sediments were mixed with the FSW then sonicated and sieved as described above. All sediments were washed from a 20µm sieve into a 45ml tubes and diluted to a suitable known volume. Living cysts were then concentrated through SPT at a specific gravity of 1.6 g cm^{-3}. Sub-samples (1ml) of processed samples were placed in a Sedgwick-Rafter chamber and cyst morphotypes counted, using an Olympus BH-2 microscope. Cyst concentrations were calculated

from known volumes of sediment suspension (cysts gram^{-1} wet sediment). A minimum of 100 cysts were counted from each sample.

2.4. Cyst Germination Experiment

Individual cysts from the SPT-recovered (1.3 g cm^{-3}) processed samples were separated by micropipette under a Leica stereo-microscope, and washed twice in growth media (GSe medium, Blackburn *et al.* 1989, see Appendix 1). Single cysts were then placed in a 55mm polystyrene petri dish containing 15ml of GSe medium, sealed with parafilm and incubated at 26°C ± 0.5°C, under cool white fluorescent light (70-90µmol photon m^{-1}s^{-1}), with a 12h light: 12h dark cycle. The remaining mixed sediment was also incubated in the same conditions. The cysts were checked regularly for germination and excysted cells isolated by micropipette and examined by LM. In addition, individual excysted cells from mix-incubated sediments were isolated by micropipette and photographed or incubated for further studies.

Established cultures of autotrophic species were transferred to flasks containing 50ml of GSe and subcultured every 4 weeks. Cultures were incubated in the same conditions as above for further experiments. Duplicate flasks of each strain were also adapted to 17°C ± 0.5°C at the same light intensity as above.

2.5. Microscopy

2.5.1. Light Microscopy

Cysts were photographed with an Olympus BH-2 light microscope equipped with a Leica DC300F digital imaging system, using bright field and phase contrast illumination. Excysted cells were stained with Calcofluor M2R fluorescent brightener and thecae structures were examined by florescence using broad band UV illumination microscopy (Fritz & Triemer 1985).

2.5.2. Scanning Electron Microscopy

Individual isolated cysts were collected on Nucleopore filters (1μm pore size) and either dried from hexamethyldisilazane (HMDL) (Nation 1983) or air dried, then sputter coated with gold (Balzers SCP004, Germany). Motile cells were concentrated by centrifuge and collected on poly-lysine coated coverslips and dehydrated in a graded methanol series (10-100 % in eight steps) and dried with (HMDL). The stubs were sputter coated with gold and examined under either a Philips Quanta 600 or JEOL JSM-840 scanning electron microscope.

3. Results

3.1 Cyst Composition and Relative Abundance

This study is the first record of dinoflagellate cysts from recent sediments of Iran's southeast coast, and provides useful information on the assemblage of dinoflagellate cysts in a tropical area. In total, forty six cyst types were identified. Table 2 shows the presence of species compared to the flora known from the Persian Gulf and the Gulf of Oman.

Table 2: Distribution of dinoflagellate cysts species in southeast coast of Iran in comparison with those from the Persian Gulf and the Gulf of Oman (Wall & Dale 1968; Bradford & Wall 1984; Marret & Zonneveld 2003)

Cyst Species	Figs	Locations				
		Bahoo-Kalat Estuary	Pasabandar	Chabahar Bay	Persian Gulf	Gulf of Oman
Gonyaulax cf. *digitale*	2		+	+	+	+
Spiniferites mirabilis	3	+	+	+	+	+
Spiniferites cf. *mirabilis*	4			+		
Gonyaulax membranaceus	5	+	+	+	+	+
Spiniferites ramosus	6		+		+	

Table 2 *continued...*

Gonyaulax baltica	7		+	+	+	+
Lingulodinium polyedrum	8		+	+	+	+
Gonyaulax sp. 1	9		+			
Gonyaulax sp. 2	10		+			
Gonyaulax sp.3	11		+			
Alexandrium cf. tamarense	12		+			+
Alexandrium cf. sp.1	13		+			
Pyrophacus steinii	14			+		+
Scrippsiella lachrymosa	15	+	+			
Scrippsiella trochoidea	16	+	+	+		
Scrippsiella irregularis	17	+	+			
Scrippsiella sp. 1	18		+			
Scrippsiella sp.2	19	+	+			
Scrippsiella cf. *trochoidea*	20	+	+			
Scrippsiella sp.3	21	+		+		
Scrippsiella sp. 4	22		+			
Protoperidinium avellana	23		+			
Protoperidinium cf. *denticulatum*	24		+			
Protoperidinium claudicans	25			+		
Protoperidinium conicum	26			+	+	+
Protoperidinium cf. *thorianum*	27			+		
Protoperidinium cf. *punctulatum*	28	+				
Protoperidinium leonis	29	+	+	+	+	+
Protoperidinium minutum	30		+			
Protoperidinium oblongum	31	+	+	+		+
Protoperidinium pentagonum	32			+	+	+
Protoperidinium cf. *conicoides*	33		+			
Protoperidinium subinerme	34			+	+	+
Protoperidinium sp.1	35			+		
Protoperidinium sp.2	36		+	+		
Protoperidinium sp.3	37			+		
Protoperidinium sp.4	38	+		+		
Protoperidinium sp.5	39			+		
Protoperidinium sp.6	40			+		
Protoperidinium sp.7	41		+	+		
Protoperidinium sp.8	42			+		
Zygabikodinium leticulatum	43			+		
Diplopsalis sp.	44			+		
Gymnodinium sp.	45	+	+			
Polykrikos kofoidii	46		+			

The most common species were: *Scrippsiella* spp., *Protoperidinium* species (*Protoperidinium oblongum, Protoperidinium leonis)*, and *Gymnodinium* sp., a previously unknown species.

The cyst assemblage in the present study was found to be primarily composed of calcareous Peridiniales, as it comprised up to 79% of cyst flora, followed by

Protoperidinium spp. (10%), *Gymnodinium* sp. (7%), Gonyaulacoid species (including *Spiniferites* spp.), *Lingulodinium* and others (2%). All other species only comprised 2% of cyst flora in the sediments (Table 3).

Cyst composition also showed some important differences between locations. *Scrippsiella* spp. and *Gymnodinium* sp. comprised 85% and 9% of cyst flora in Pasabandar. Over 85% of cyst flora of Bahoo-Kalat estuary was *Scrippsiella* species, while 4% of cysts were *Gymnodinium* sp. Chabahar Bay clearly differed from the two other areas as *Protoperidinium* spp. was the dominant group with 51% of cyst flora being *Protoperidinium* spp.; of these, round brown cysts of *Protoperidinium* (*Protoperidinium* sp. 7) *P. oblongum*, *P. pentagonum* were the most common species (see Appendix 1, Table 1). *Scrippsiella* comprised only 38% of the cyst flora in this bay. Gonyaulacoid cysts including *L. polyedrum* also increased from 1% in both Bahoo-Kalat estuary and Pasabandar to 7% in Chabahar Bay (Table 3). Highest cyst concentrations were found in Pasabandar (6479 cyst/gram wet sediment) and lower concentrations recorded in Bahoo-Kalat estuary (494 cyst/gram wet sediment). Cyst concentrations of Chabahar bay were 1214 cyst/gram wet sediment (Table 3).

Table 3: Relative (% cyst) abundance and total number of cyst at each location and all sites (Cyst/g wet weight)

Species \ Locations	Bahoo-Kalat Estuary	Pasabandar	Chabahar Bay	All Sites
Protoperidinium spp.	4	3	51	10
Scrippsiella spp.	85	85	38	79
Gonyaulax spp.	1	1	7	2
Gymnodinium sp.	4	9	0	7
Others (mainly *Alexandrium, Polykrikos, Zygabikodinium*	6	2	4	2
Total number of cysts (cyst/g wet weight)	494	6479	1214	

3.2. Systematics

In this study, the biological names of the species are used and palaeontological names are given, where possible. In the following, a brief description of the cysts, the

distribution of the cyst types in adjacent tropical/subtropical areas (Indian ocean, Arabian Sea, Gulf of Oman and Persian Gulf), and a list of references used in identification of the cysts are given. The systematic account presented in this survey follows the classification of Fensome *et al.* (1993).

Phylum: Dinophyta
Class: Dinophyceae Pascher
Order: Gonyaulacales Taylor
Family: Gonyaulacaceae Lindemann
Genus: *Gonyaulax* Diesing
"Spinifera Group" Kofoid

***Gonyaulax spinifera* complex**

Five "Spinifera group" cyst types were clearly identified from the samples collected. Their palaeontological names are: *Spiniferites bentori*, *S. mirabilis*, *S. membranaceus*, *S. bulloideus* and *S. ramosus*. The cyst-theca relationship between *S. bentori* and *G. digitale* was uncertain in the study of Lewis *et al.* (2001). They linked *Bitectatodinium tepikiense* Wilson to the *Gonyaulax digitale* (Pouchet) Kofoid, instead of *S. bentori* (Rossignol).

References: Wall & Dale (1968); Dale (1983); Bolch & Hallegraeff (1990); Ellegaard *et al.* (2002); Ellegaard *et al.* (2003).

Gonyaulax* cf. *digitale (Pouchet) Kofoid **Figs 2a-b**

Synonym: *Protoperidinium digitale* (Pouchet)
[Palaeontological taxon: S*piniferites bentori* (Rossignol) Wall & Dale, *Bitectatodinium tepikiense* Wilson]

An ovoid cyst with bifurcate and trifurcate processes. Cyst length 42-47μm, width 38-42μm, processes 8-14μm in length. A paracingulum is indicated by two parallel ridges above the equatorial region of the cyst; interconnected parasaturals define the paracingular region (Figs 2a-b). The archeopyle is precingular. Viable cysts contain red accumulation body and globular contents. None were successfully germinated.

Only a few cysts were found in sediments. The cyst was also rare in the northern Arabian Sea (the Gulf of Oman) (Zonneveld 1997) but was common in recent sediment from the Persian Gulf with salinity of over 40ppt (Bradford & Wall 1984). The cyst has longer processes and a more elongated body compared with those described for *G.* cf. *digitale* (round cyst) by Sonneman and Hill (1997), and Wall and Dale (1968). The long spines and cyst outline is similar to cysts distinguished by Bradford and Wall (1984) from the Persian Gulf.

References: Dale (1983), Fig. 5; Orlova *et al.* (2004), Figs 18-19; Ellegaard *et al.* (2003), Figs 50-54.

Spiniferites mirabilis (Rossignol) Sarjeant **Fig. 3**

A spherical, light brown to colourless, two-layered cyst. Cyst diameter 40-45μm, processes 9-15μm. Tabular or conical processes are bifurcated or trifurcated; some are fused, particularly in the antapical area and form a distinct flange (Fig. 3). The precingular archeopyle was not observed. Viable cysts with globular contents were found, but none were successfully germinated.

This cyst type was smaller in size compared with the larger ovoid to spherical cyst described by Bolch & Hallegraeff (1990), and Sonneman & Hill (1997) from

Tasmanian and Victorian coastal sediments of Australia. The size and shape are similar to those described from the Russian Pacific coast described by Orlova *et al.* (2004). The size is also comparable with those from the Persian Gulf (Bradford & Wall 1984) that are slightly smaller in size (37μm width and 42μm length).

The cyst was not very common in the study area. It was known also from sediment trap material from the Arabian Sea (southeast of the Gulf of Oman) but was not common (Marret & Zonneveld 2003). It was, however, widespread in the Persian Gulf (Bradford & Wall 1984). In addition, the cyst was rare in southwest Indian coastal sediment (Godhe *et al.* 2000). There is no morphological description from these tropical areas to compare with *S. mirabilis* from southeast coast of Iran.

References: Bolch & Hallegraeff (1990), Fig. 4; Joyce (2004), Fig. 3; Sonneman & Hill (1997), Fig. 4a; Orlova *et al.* (2004), Fig. 24-25.

Spiniferites* cf. *mirabilis **Figs 4a-b**

Spherical small cyst with light brown transparent colour. Cyst diameter is 29μm with 7-8μm processes. The processes are tabular, with bifurcate or trifurcate tips. Some processes connected at distal tips with W-shaped extremities (Fig. 4). No archeopyle was observed. Only one specimen was found in sediment, and a germination experiment was not carried out.

The cyst differs from *S. mirabilis* (this study) by its smaller size. The cyst is similar in W-shaped process to that described by Bolch and Hallegraeff (1990) from Tasmania/Australian sediment (Fig. 4c). The size is smaller than that of Persian Gulf identified by Bradford and Wall (1984, Plate 4, Fig. 3) and there is no size range reported for *S. mirabilis* from other adjacent tropical areas (the Gulf of Oman and other parts of the Arabian Sea). In other temperate areas, *S. mirabilis* varies from 58-70μm in length and 45-58μm in width (Bolch & Hallegraeff 1990; Sonneman & Hill 1997; Joyce 2004a).

Gonyaulax membranaceus (Rossignol) Ellegaard, Daugbjerg, Rochon, Lewis, Harding **Figs 5a-b**

Synonym: *Spiniferites membranaceus* (Rossignol) Sarjeant

A spherical to ovoidal, transparent cyst. Cyst length 42-44μm, width 37-40μm, processes 13-20μm. The processes are membranous, particularly in the antapical area, and trifurcate with broad bifid tips (Figs 5a-b). The quadrangular precingular archeopyle was not observed. None were successfully germinated.

This cyst type is slightly larger than those described by Bolch and Hallegraeff (1990), Sonneman and Hill (1997) and Ellegaard *et al.* (2003). However, a size range of 37-50μm (width) and 41-54μm has been reported for the species (Marret & Zonneveld 2003). This cyst type has previously been recorded from the central part of the Persian Gulf, Straits of Hormuz and some coastal areas of the Arabian Sea (Bradford & Wall 1984) and southwest of India (Southeast Arabian Sea) by Godhe *et al.* (2000).

References: Bolch & Hallegraeff (1990), Fig. 3; Sonneman & Hill (1997), Fig. 4c; Lewis *et al.* (1999), Figs 1-19; Ellegaard *et al.* (2003), Figs 31-45.

Spiniferites ramosus (Rossignol) **Fig. 6**

A spherical cyst, pale-brown in colour. Cyst diameter 42μm, processes 11-19μm. The cyst is ornamented by trifurcate processes with bifid tips. The cyst is characterised by two pronounced antapical processes (Fig. 6). Only one specimen was found in sediment and germination was unsuccessful.

This cyst has been reported from the southern Gulf of Oman, in the coastal area of Muscat and Oman, in the northern Arabian Sea and the Persian Gulf (Bradford & Wall 1984). High relative abundance of the species has been reported from the Arabian Sea (Marret & Zonneveld 2003).

References: Bradford & Wall (1984), Plate 4, Figs 15-17; Joyce (2004b), Figs 2a-b.

Gonyaulax baltica Ellegaard, Lewis & Harding **Figs 7a-d**

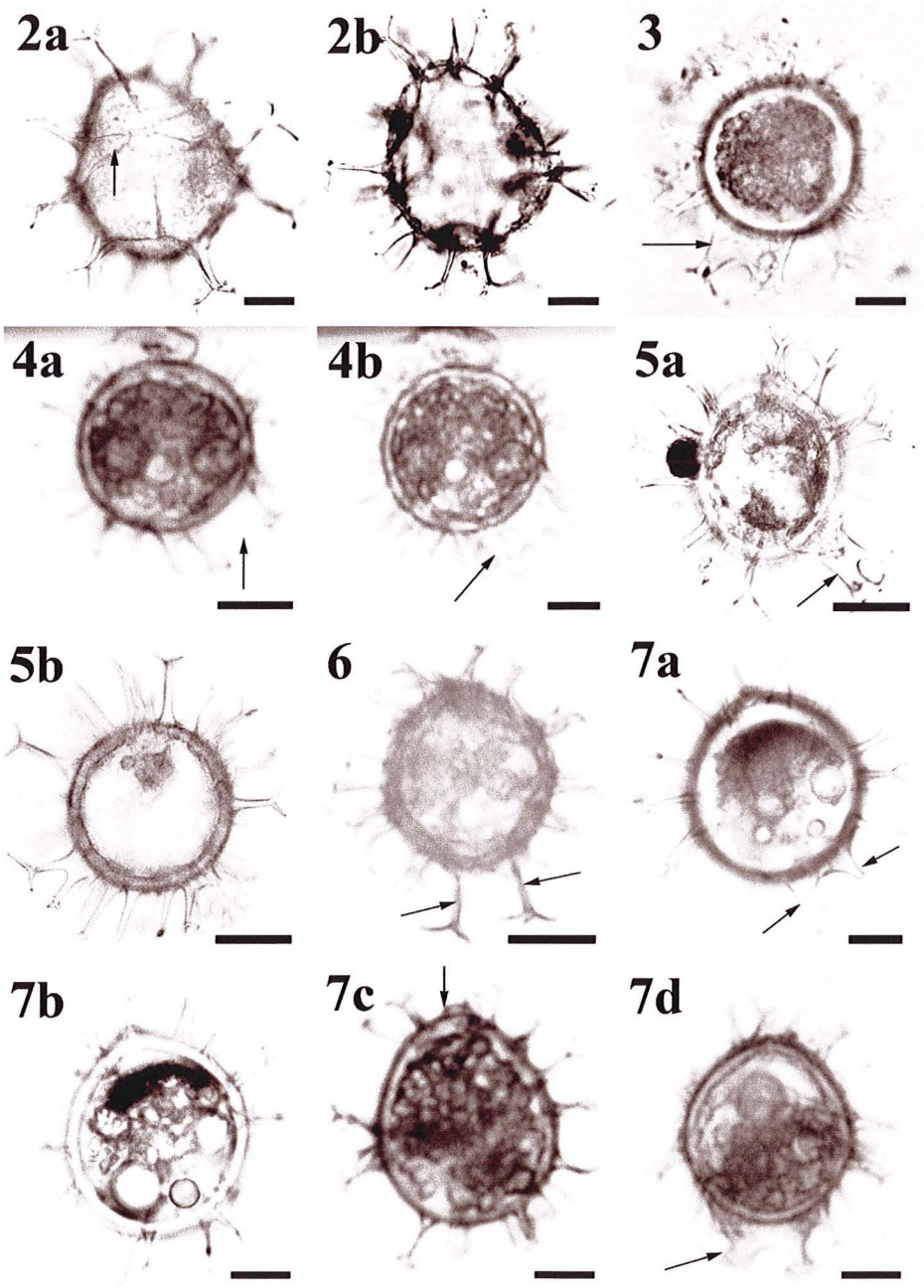
A small sub-spherical to ovoidal cyst with a smooth surface. Cyst length 31-42μm, width 30-36μm, processes 7-12μm. The processes are trifurcate with bifid tips. A membranous flange process in antapical area expands into two distinct columnar stems (Fig. 7a). An apical boss is sometimes present (Figs 7b-c). Trumpet-shaped processes (Fig. 7d) are similar to those described by Ellegaard *et al.* (2003). The cysts contain an orange accumulation body and globular content. The archeopyle is precingular and in the dorsal surface but was not observed in this study. None were successfully germinated.

This cyst type showed variation in process length and shape (Figs 7a-d). Some were similar to those known as *Gonyaulax scrippsiae* Kofoid (=*Spiniferites bulloideus* (Deflandre & Cookson) Sarjeant) as described by Sonneman and Hill (1997). However, the trumpet-shaped processes developed septa and presence of an apical boss more resembled *G. baltica*. Ellegaard *et al.* (2002) separated cysts of *G. baltica* from S. *bulloideus* by their wide range of variation in process length and morphology and also the degree of development of septa found in *G. baltica*. The motile stage of *G. baltica* has been found to clearly differ from *G. scrippsiae* (Ellegaard *et al.* 2002). As none of our cysts were successfully germinated, we can not be certain of the identity of this cyst. *S. bulloideus* has been reported from the Gulf of Oman and the Arabian Sea in low numbers (Bradford & Wall 1984; Marret & Zonneveld 2003).

References: Sonneman & Hill (1997), Figs 3a-b; Ellegaard *et al.* (2003), Figs 1-5.

Plate 1: Gonyaulacoid resting cysts from the southeast coast of Iran

Figs 2a-b. LM. *Gonyaulax* cf. *digitale.*
Fig. 2a. Cyst showing paracingulum region and granular surface (arrow).
Fig. 2b. Same cyst in deep focus.
Fig. 3. LM. *Spiniferites mirabilis*, cyst showing flange like antapical process.
Figs 4a-b. LM. *Spiniferites* cf. *mirabilis*, cyst showing a W-shaped process (arrow).
Figs 5a-b. LM. *Gonyaulax membranaceus*, cysts showing relatively long processes and antapical flange.
Fig. 6. LM. *Spiniferites ramosus* with identical antapical processes.
Fig. 7a-d. LM. *Gonyaulax baltica.*
Fig. 7a. Cyst showing a membranous flange process (arrow).
Fig. 7b. Cyst with globular content.
Fig. 7c. Cyst showing an apical boss and trifurcate processes with bifid tips.
Fig. 7d. Cyst showing a trumpet-shaped process (arrow).
All scale bars=10μm, except for Figs 5-6 scale bars=20μm.



Lingulodinium polyedrum (Stein) Dodge **Figs 8a-h**

Synonym: *Gonyaulax polyedra* (Stein)
[Palaeontological taxon: *Lingulodinium machaerophorum* (Deflandre & Cookson) Wall]

A colourless, spherical cyst with two-layered wall, 32-47μm diameter. The cyst wall appears to be granular and thick (Figs 8a-c). The cyst is ornamented with hollow tabular and non-tabular processes of variable length (2-9μm). The processes are large with a weak irregular shaped base, which tapers to the tabular spine (Figs 8a-e). We did not observe the archeopyle, however a large compound archeopyle has been reported by Lewis and Hallett (1997) consisting of one to five precingular paraplates. The theca of the germinated cell has strong sutures along the paraplate. The first apical plate is long and has a ventral pore on its right side (Fig. 8g). The 6" precingular plate is pentagonal (Fig. 8h). The hypotheca has a flat antapex, without any projection (Fig. 8f).

This cyst type was the most common Gonyaulacoid cyst. Some cysts were successfully germinated and established unialgal cultures. Several cells were isolated to form the clonal cultures but none of them survived. This cyst has been previously reported from the north-western part of the Gulf of Oman and Kuwait coastal sediment (Persian Gulf) (Bradford & Wall 1984; Husain & Lewis 2004). In addition, the cysts were found in very low amounts in the Arabian Sea, and the southwest Indian coast (Marret & Zonneveld 2003, Godhe *et al.* 2000).

References: Wall & Dale (1968), Plate1, Figs 17-18; Lewis & Hallett (1997), Figs 11-25; Joyce (2004b), Plate 1, Figs 6a-6d.

***Gonyaulax* sp. 1** **Fig.9**

An ovoid cyst with a dark brown wall. Cyst length is 38μm and width 31μm.The processes are trifurcated, with bifid tips and 9-11μm in length. This cyst species is

Genus: ***Alexandrium*** Halim

Alexandrium* cf. *tamarense (Lebour) Balech **Figs 12a-b**

A cylindrical cyst with rounded ends. Cyst length 32-44μm, width 25-37μm. The cyst has a two-layer wall covered with transparent mucilaginous material and adherent fine particles (Figs 12a-b). The cyst contents are composed of colourless starch and lipid globules and a bright orange accumulation body (Fig. 12a).

Three cysts of this type were found in Iranian sediment; none were successfully germinated to confirm the identification of this type. A similar cyst type with very low relative abundance, but a larger size (45-48μm in length) has been also recorded from the southwest Indian coast (Godhe *et al.* 2000). There is no other record of this cyst type from the Gulf of Oman and the Arabian Sea.

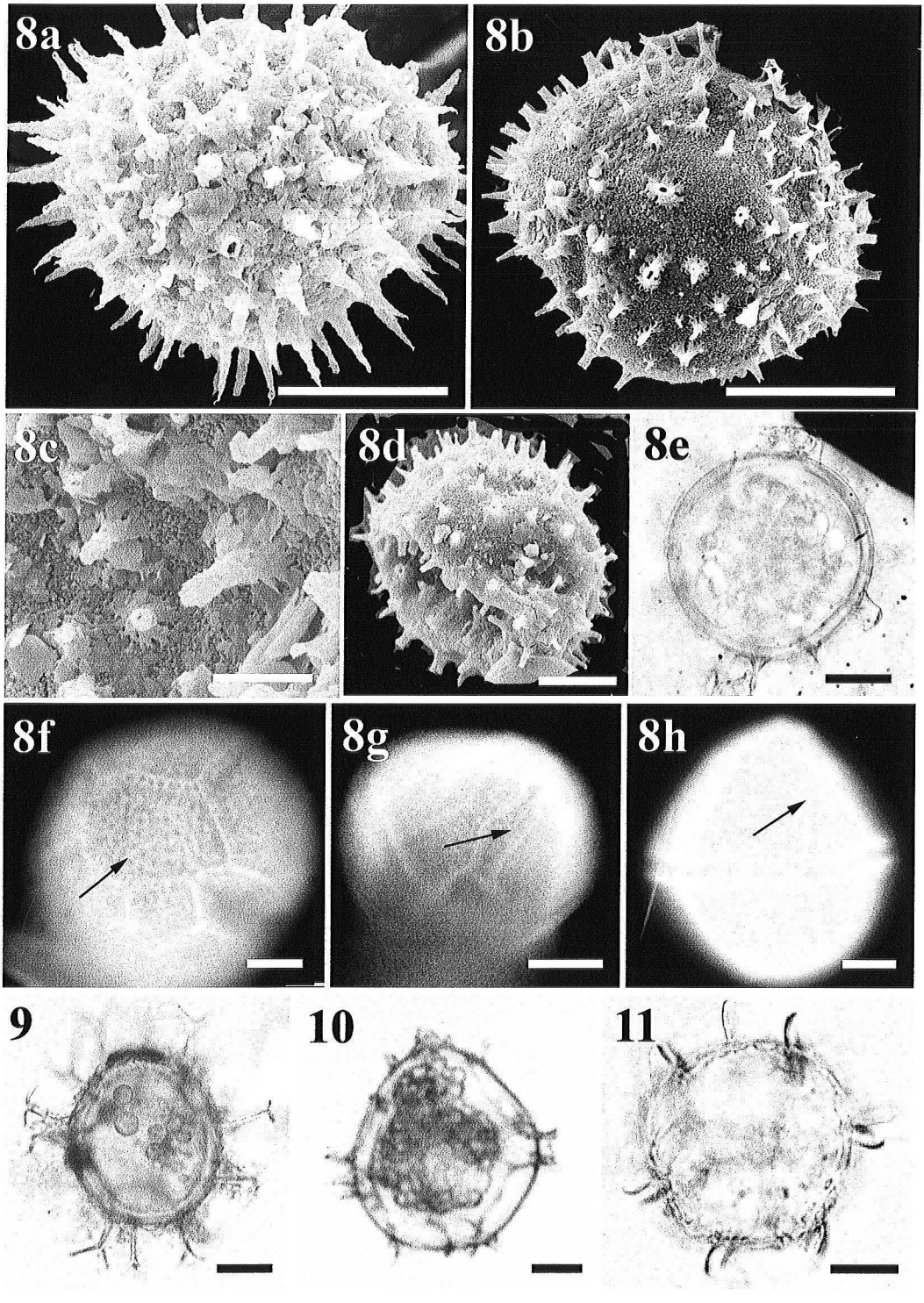
References: Bolch & Hallegraeff (1990), Fig. 7; Qi *et al.* (1996), Plate 1, Fig. A; Dongzhao *et al.* (2003), Plate 1, Fig. 4; (2004b), Fig. 2.

***Alexandrium* cf. sp. 1** **Fig. 13**

A cylindrical cyst with generally rounded ends. Cyst length 54μm, width 41μm. The cyst wall is covered with a mucilaginous layer with adherent detritus particles. The cyst has globular contents and an orange-red accumulation body. The cyst was not successfully germinated.

Plate 2: Gonyaulacoid resting cysts and thecae from the southeast coast of Iran

Figs 8a-h. *Lingulodinium polyedrum,* Cyst shows variety in process length.
Fig. 8a. SEM. Cyst showing long hollow process.
Fig. 8b. SEM Cyst with short tabular processes.
Figs. 8c-d. SEM. Processes with a weak base.
Figs 8e. LM. Cyst showing globular content.
Fig. 8f. Cell Antapical view of the Calcofluor-stained vegetative cell indicates the fist antapical plate (arrow).
Fig. 8g. Ventral view of Calcofluor-stained cell represents the first apical plate (arrow).
Fig. 8h. Lateral-dorsal view of Calcofluor-stained cell showing apical plates and the 6" pentagonal precingular plate (arrow).
Fig. 9. LM. *Gonyaulax* sp.1 cyst showing dark colour.
Fig. 10. LM. *Gonyaulax* sp. 2 Cyst showing an apical boss and short radial processes.
Fig. 11. LM. *Gonyaulax* sp. 3. Cyst showing paracingulum region (arrow).
All scale bars=10μm except for Figs 8a-b=20μm, Fig. 8c=5μm, Fig. 8f=5μm.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11

Family: Pyrophacaceae Lindemann

Genus: *Pyrophacus* Stein

Pyrophacus steinii (Schiller) Wall & Dale **Fig. 14**

Synonym: *Pyrophacus vancampoae* (Rossignol)

[Palaeontological taxon: *Tuberculodinium vancampoae* (Rossignol) Wall]

A large disk-shaped cyst with an outer transparent membrane. The cyst is ornamented with short dumb-bell shape tubercules. Cyst length 76-85μm width 56-75μm and tubercules 10-15μm. This cyst has two separate wall layers, with tubercules positioned between them, around a central body. The central body is compressed anterior-posteriorly; therefore the apical and antapical views are subcircular to reniform and the lateral view is rectangular, with somewhat rounded corners. According to Fensome *et al.* (1993), the archeopyle is subtrapezoidal in shape and hypocystal; it is formed by the loss of 3-5 antapical plates.

Only two were found in sediments; none were successfully germinated. The cyst is more rounded compared with those described by Sonneman and Hill (1997). The cyst shape more resembles that described from the Gulf of California (subtropical area) by Morquecho and Lechuga-Deveze (2003).

This species is also known from the Persian Gulf with high abundance near the Iranian coastline; while it is present in low abundance in the northern part of the Arabian Sea (Bradford & Wall 1984).

References: Fensome *et al.* (1993), Fig. 111; Sonneman & Hill (1997), Figs 10a-b. Morquecho & Lechuga-Deveze (2003), Figs 14-15.

Order: Peridinales Haeckel
Family: Peridiniaceae Ehrenberg
Genus: *Scrippsiella* (Balech) Loeblich III

Scrippsiella lachrymosa Lewis **Figs 15a-b**

Elongated oval cyst with a light purplish colour. The cysts are 31-35μm in length and 24-26μm in width. The outer wall is covered with thin, flattened, and calcareous plates (Fig. 15a). There are gaps between the plates. A red-orange accumulation body can be observed in the middle or anterior part of the cyst (Figs 15a-b). The archeopyle was not observed. The cyst was not common. None were successfully germinated.

Lewis (1991) found that in some specimens the calcareous crystals start to overgrow each other, however, we did not observe overlaps in the plates. Some specimens were found without calcareous crystals. The species has not been reported previously from the Gulf of Oman or the Arabian Sea.

References: Lewis (1991) Figs 30-36; Orlova *et al.* (2004), Figs 68-69.

Scrippsiella trochoidea (Stein) Loeblich III **Figs 16a-f**

Synonym: *Peridinium trochoideum* (Stein) Lemmermann, *Peridinium faeroense* Paulsen, *Glenodinium trochoideum* Stein, *Glenodinium acuminatum* Jorgensen, *Scrippsiella faeroense* (Paulsen) Balech & Soares.

A dark brown cyst; spherical, oblate or oval in shape. Cyst diameter 21-32μm and cyst length 28-38μm. This cyst was highly variable in size, shape and surface features. The calcareous processes have polygonal base plates and a three cornered pointed tip (Figs 16e, g, & h). The processes may be long (Fig. 16a), short (Fig. 16b)

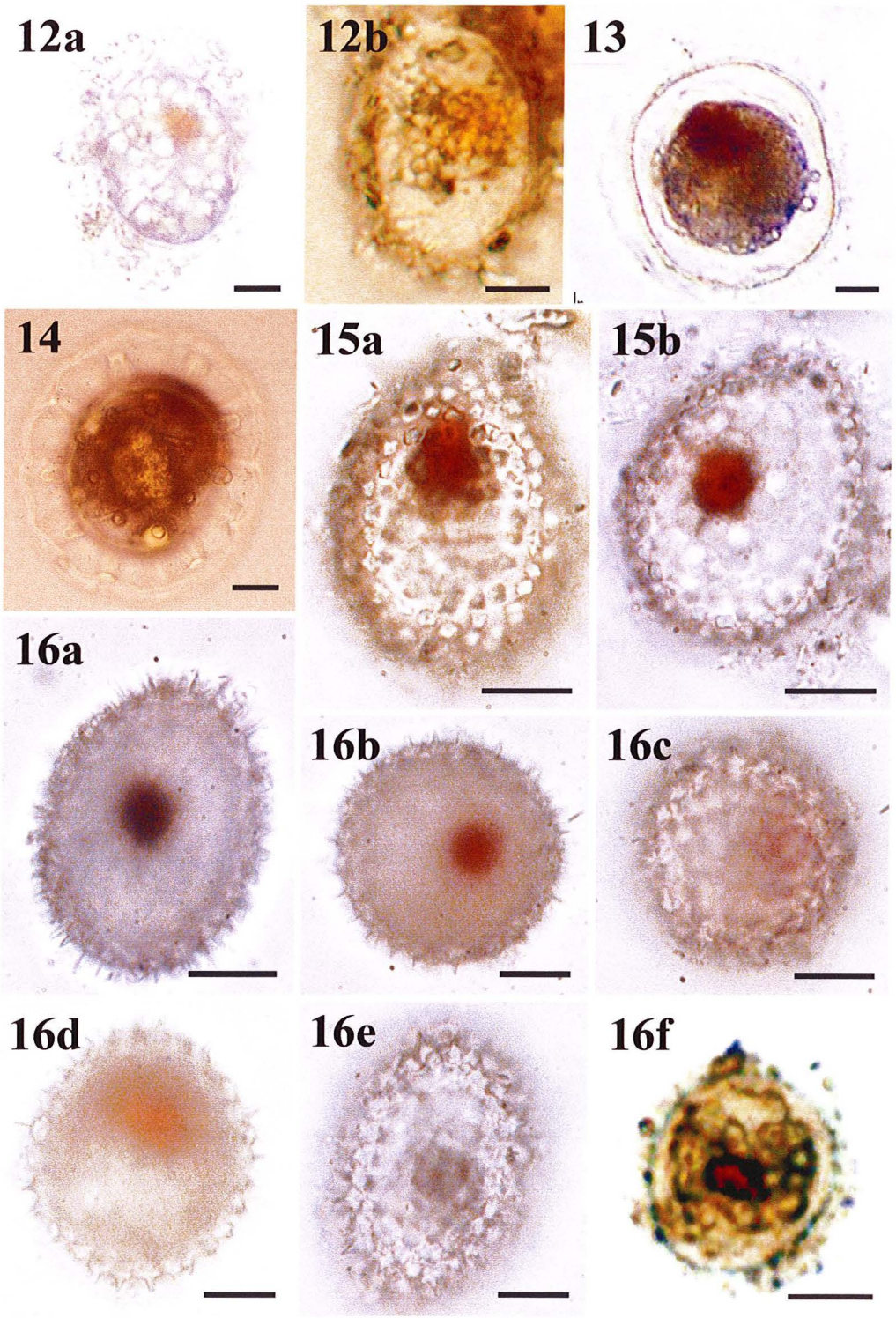
or the cyst may be without any spines (Fig. 16f). Viable cysts contained a distinct red accumulation body (Figs16a-f). The cyst has a theropylic archeopyle that was observed in cultured cysts (Fig. 16c).

This cyst was widespread in Iranian sediments. Many cysts were successfully germinated to produce typical *Scrippsiella* cells (Fig. 16e), matching the description of *S. trochoidea*. However, molecular studies (see Chapter 4) suggest four distinct genotypes in this group.

References: Bolch & Hallegraeff (1990), Fig. 11; Lewis (1991), Figs 1-7; Janofske (2000), Figs 8-14.

Plate 3: Resting cysts of *Alexandrium, Pyrophacus* and *Scrippsiella* from southeast coast of Iran

Figs 12a-b. LM. *Alexandrium* cf. *tamarense*, Cyst with enclosed a mucilaginous layer, shows lipid globules contents and orange accumulation body.
Fig. 13. LM. *Alexandrium* cf. sp.1 cylindrical cyst with adherent mucilaginous layer.
Fig. 14. LM. *Pyrophacus steinii*, epicystal view indicating central body surrounded by dumbbell shape process and membranous outer wall layer.
Fig. 15a-b. LM. *Scrippsiella lachrymosa,* cyst showing the calcareous plates.
Fig. 16. *Scrippsiella trochoidea* cyst shows variety in shape and process.
Fig. 16a. LM. Oval cyst with red accumulation body.
Fig. 16b. LM. Spherical cyst of same species.
Fig. 16c. LM. Surface focus on the cyst showing the process base.
Fig. 16d. LM. Sub-spherical cyst.
Fig. 16e. Same cyst in surface focus showing the irregular base.
Fig. 16f. LM. Cyst without any processes.
All scale bars=10μm.


12a
12b
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16b
16c
16d
16e
16f

***Scrippsiella irregularis* sp. nov. Attaran-Fariman & Bolch** **Fig 17a-e**

A spherical to oval cyst with a light grey to light brown colour. Cyst diameter is 20-26μm. Viable cysts have light globular contents and a red-orange accumulation body. Cyst is ornamented with numerous pointed calcareous spines (Figs 17a, c). The original cyst germinated to produce live cells was without spines and covered with an irregular mucilaginous layer (Fig. 17b). Two cysts were successfully germinated to produce *Scrippsiella irregularis* (Fig. 17e) (see Chapter 4). Cysts were also produced in unialgal cultures (Fig. 17d).

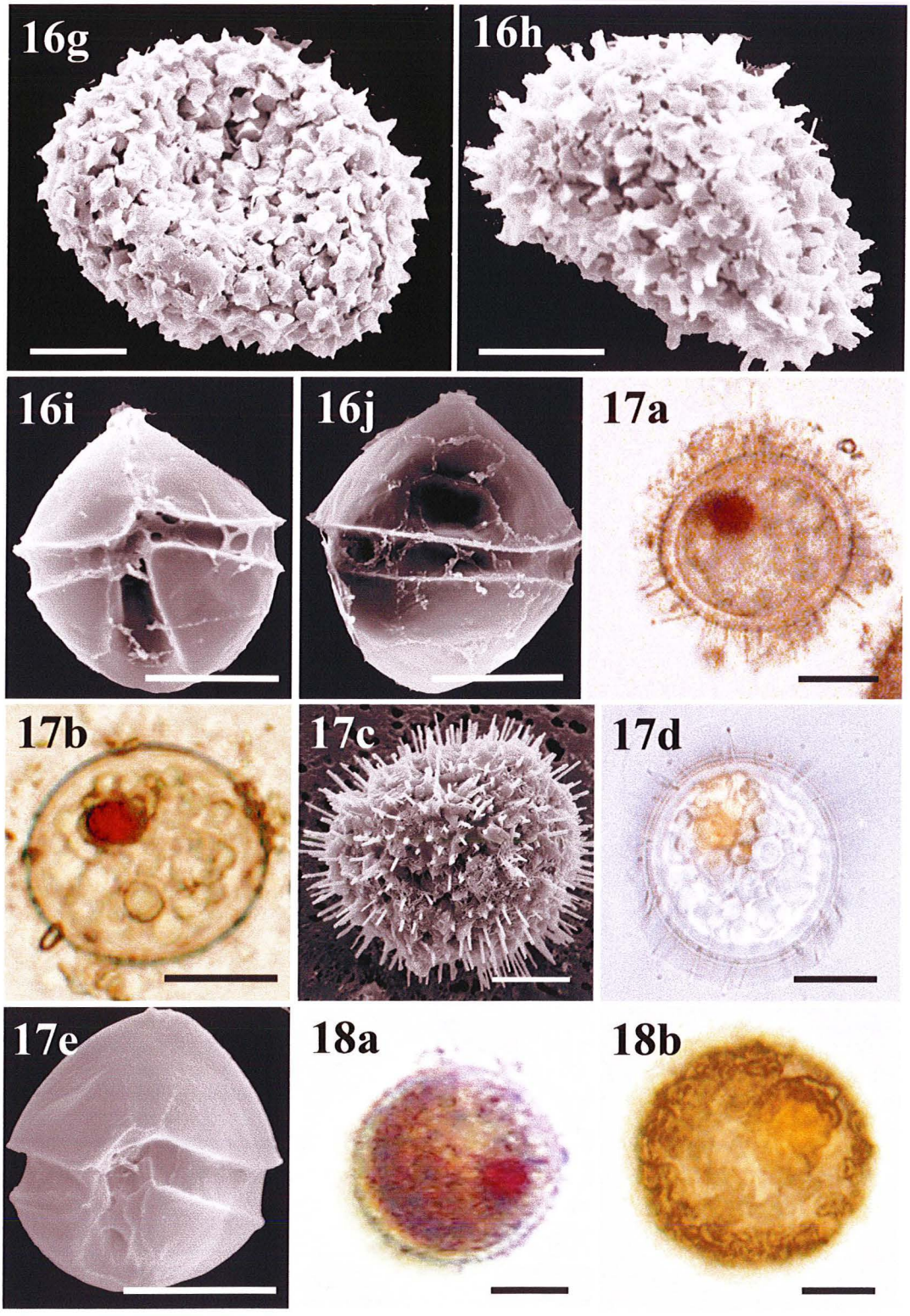
This cyst type is very similar to the cyst of *S. precaria* Montresor & Zingone in shape of the cyst and processes but is slightly larger in size. However, molecular studies and morphological analysis of the motile cells suggest that it is a quite distinct new species (see Chapter 4). This cyst was common in the sediment of only one location (Bahoo-Kalat estuary). There is no report of this species from adjacent tropical regions.

Reference: Montresor & Zingone (1988) Figs 1-8

***Scrippsiella* sp.1** **Fig 18a-d**

Spherical to subspherical cyst with a dark brown colour. Cyst diameter 27-37μm, without any distinct ornamentation on the cyst wall (Fig. 18a). Two cysts were successfully germinated to release *Scrippsiella* cells (Fig. 18d). Cysts were produced in unialgal cultures that were covered with calcareous plates (Fig. 18b); however, a few cysts were surrounded by spines (Fig. 18c). This cyst is differed from *S. trochoidea* in its process, shape and colour (see Chapter 4 for detail). Molecular data suggest this species is closely related to *Scrippsiella trochoidea* var. *aciculifera.* However, the motile stage of this species is distinct from *Scrippsiella trochoidea* var. *aciculifera* due to lack of spine on its anterior sulcal plate (see Chapter 4). No archeopyle was observed.

References: D' Onofrio *et al.* (1999), Figs 30-38; Montresor *et al.* (2003), Figs 17-28.


16g
16h
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18b

***Scrippsiella* sp. 2** **Figs 19a-d**

A large spherical to subspherical cyst with a green to brown colour. Cysts varied in size from 39-64μm. Cyst contains green (in wild cyst, Fig. 19a) or grey (in cultured cysts, Fig. 19b) globular contents, and also a red accumulation body. The cyst has a thin calcareous outer wall with a reticulate crystalline structure (see Chapter 4). No paratabulation is clear on the cyst wall (Figs 19b-d).

This cyst type is similar in size to *Scrippsiella sweenyae* (Balech) Loeblich, described by Wall and Dale (1968) from the western Arabian Sea. However, they are different in cyst outline as *S. sweenyae* has an irregular shape under the light microscope due to paratabulation on the outer wall (Wall & Dale 1968, Figs 5-6) and also possesses a microgranular texture in the outer wall. In contrast, *Scrippsiella* sp. 2 has a spherical shape, smooth outer wall with reticulate pattern that is without paratabulation (see Chapter 4 for more detail). This cyst was widespread in Iranian sediments, and one cyst was successfully germinated to produce *Scrippsiella* cells (Fig. 19c).

Reference: Wall & Dale (1968), Figs 4-7

Scrippsiella* cf. *trochoidea **Figs 20a-c**

Round to ovoid cysts, brown in colour. Cyst diameter 18-37μm. The original cyst that established the culture was without clear ornamentation (Fig. 20a). Five cysts germinated to produce *S. trochoidea* like species (Fig. 20b). Spiny calcareous cysts were produced in cultures, similar to cysts of *S. trochoidea* (Fig. 20c). Molecular study and morphological analysis suggest that this species is closely related to *S. trochoidea* (see Chapter 4, referred to as *S. trochoidea*).

***Scrippsiella* sp. 3** **Figs 21a-c**

Spherical, dark green-brown cysts ornamented with evenly distributed conical calcareous processes. Cyst diameter is 38-41μm. The outer cyst wall is clear and has a darker colour, ornamented with distally pointed processes (Figs 21a-c), the inner layer possesses a light colour. A red accumulation body and globular lipid contents are present.

This cyst type was common, however, none were successfully germinated and none of my cultures produced cysts matching this morphology. The cyst differs from *S. trochoidea* and *Scrippsiella* cf. *trochoidea* by its colour, larger size and distinctive conical processes.

***Scrippsiella* sp. 4** **Figs 22a-c**

Oval to oblate cysts covered by calcareous crystals. Cyst length 33-35μm and width 25-28μm. The cysts are ornamented with irregular or rounded crystals with gaps between them (Figs 22a-d).

These cysts were isolated directly from processed sediment and prepared for SEM, therefore no germination experiments were carried out.

Montresor *et al.* (2003) found cysts of *S. trochoidea* (strain SZN72) that were spherical and covered with club-shaped calcareous crystals. Cysts of *Scrippsiella* sp. 4 differ from those described by Montresor *et al.* (2003) by cyst shape and the irregular or rounded crystals rather than polygonal crystals.

Reference: Montresor *et al.* (2003), Fig. 27

Plate 5: *Scrippsiella* spp. resting cysts and germinated thecae from southeast coast of Iran

Fig. 18c. LM. *S.* cf. *trochoidea* spiny cyst produced in culture.
Fig. 18d. Calcofluor-stained vegetative cell germinated from this cyst type.
Figs19-a-d. LM. *Scrippsiella* sp. 2.
Fig. 19a. Large cyst with reticulate pattern isolated from sediment.
Fig. 19b. Cyst with reticulate pattern produced in culture.
Figs 19c. Calcofluor-stained vegetative cell germinated from this cyst type.
Fig. 19d. Cultured cyst with partially attach outer calcareous wall.
Figs 20a-c. LM. *Scrippsiella* cf. *trochoidea.*
Fig. 20a. Cyst without any process germinated to produce a *Scrippsiella* cell.
Fig. 20b. Calcofluor-stained germinated vegetative cell in ventral view.
Fig. 20c. Spiny cyst produced in culture showing the archeopyle.
Figs 21a-c. LM. *Scrippsiella* sp. 3.
Fig. 21a. Cyst showing dark outer wall and accumulation body.
Fig. 21b. Cyst showing process with pointed tip and wider bases.
Fig. 21c. Cyst showing globular content.
All scale bars=10μm.

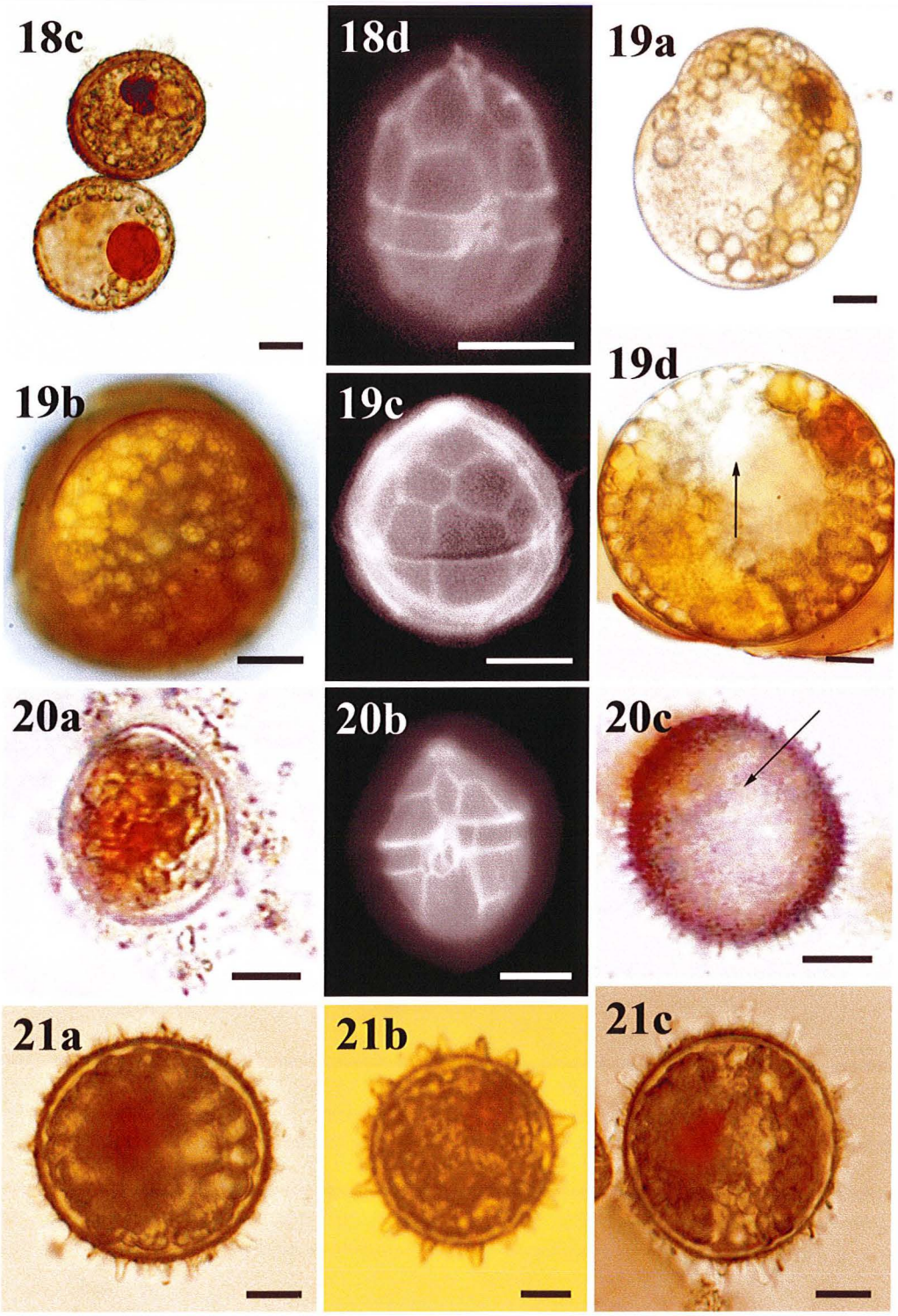
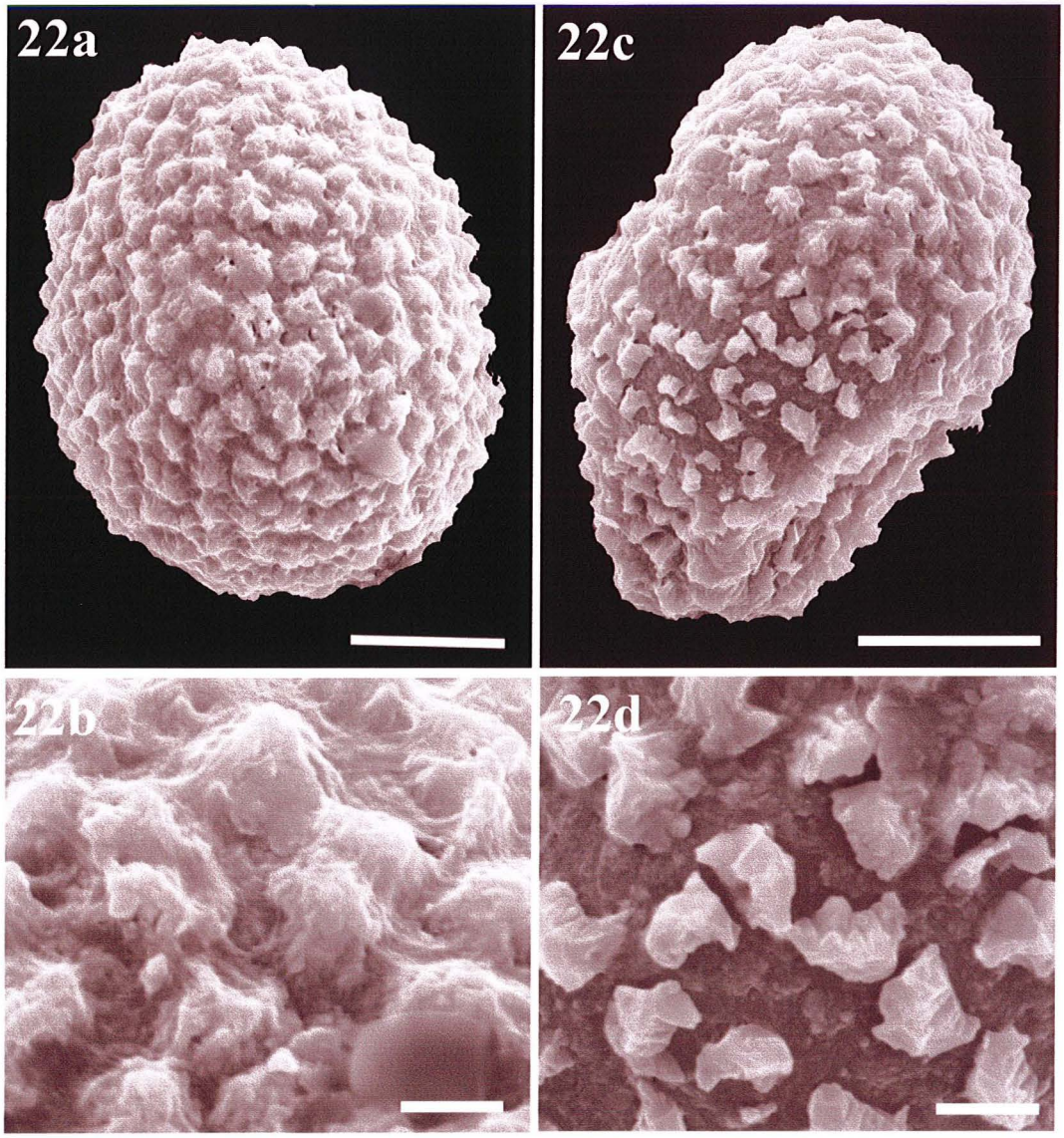

18c
18d
19a
19b
19c
19d
20a
20b
20c
21a
21b
21c

Plate 6: *Scrippsiella* resting cysts from southeast coast of Iran

Fig. 22. SEM. *Scrippsiella* sp. 4.
Fig. 22a. Cyst showing rounded plates.
Fig. 22b. Details of cyst surface.
Fig. 22c. Oblate cyst showing irregular shaped crystals.
Fig. 22d. Detail of cyst surface showing gaps between crystals.
Scale bars=10μm in Figs 22a and 22c, scale bars=5μm in Figs 22b, 22d.


22a
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Family: Protoperidiniaceae (Bujak) Davies

Genus: *Protoperidinium* (Bergh) Balech

Protoperidinium avellana (Meunier) Balech **Fig. 23**

Synonym: *Protoperidinium avellana Meunier, Peridinium avellana* Lebour
[Palaeontological name: *Brigantedinium cariacoense* (Wall) Reid]

A spherical cyst with a dark brown colour (diameter 52μm). The cyst has a smooth and single layer wall. A symmetrical hexagonal archeopyle appears to be formed by loss of one intercalary paraplate (1a).

Only one empty cyst was found in sediments. The shape of the archeopyle in the cyst is similar to that of Joyce's studies (2004a, 2004b) from Scottish and South African coastal sediment. The archeopyle shape can be variable in *P. avellana* cysts, reflecting loss of either one or two intercalary plates. Cysts with archeopyl which reflect the loss of two paraplates have been recorded previously by Sonneman and Hill (1997) from Australian sediments. This cyst type was also reported from the Arabian Sea and southwest India (Marret & Zonneveld 2003; Godhe *et al.* 2000), but there is no morphological description of the species in these works.

References: Wall & Dale (1986), Plate 3, Fig. 29; Sonneman & Hill (1997), Fig. 13; Joyce (2004b), Fig. 13.

Protoperidinium* cf. *denticulatum (Gran & Braarud) Balech **Fig. 24**

Synonym: *Peridinium denticulatum* (Gran & Braarud)
[Palaeontological name: *Brigantedinium irregulare* (Matsuoka)

A brown spherical cyst with a smooth wall. Cyst diameter 41-42μm. The cyst has an elongate, asymmetrical and hexagonal archeopyle, formed by loss of the two intercalary (1a, 2a) paraplates.

Two empty cysts were found. The subhexagonal archeopyle in this type differs from the lunate-shaped archeopyle described for *P. denticulatum* (Sonneman & Hill 1997; Wang *et al.* 2004; Joyce 2004b).

References: Sonneman & Hill (1997), Fig. 14c; Wang *et al.* (2004), Fig. k; Joyce (2004b), Figs 2a-d.

Protoperidinium claudicans (Paulsen) Balech **Fig. 25a-d**

The motile cell of *P. claudicans* was germinated and isolated from mixed-incubated sediment. The pentagonal body of the motile cell is broadly rounded along the girdle. The concave conical epitheca tapering to an apical horn (Figs 25b-c). The cell has a four-sided first apical plate (Fig. 23d). The hypotheca is pointed, with two diverging conical antapical horns (Figs 23a, d). The left antapical horn is shorter than the right one (Fig. 23c). Cysts of *P. claudicans* are heart-shaped, 52-57μm long, light brown and ornamented with numerous short pointed spines (Bolch & Hallegraeff 1990; Morquecho & Lechuga-Deveze 2003; Orlova *et al.* 2004; Joyce 2004a). But, such a spiny empty cyst that released the motile cell in the mixed-incubated sediment was not found.

References: Wall & Dale (1968), Plate 1, Fig. 30 and Plate 3, Fig. 12. Sonneman & Hill (1997), Fig. 18c.

Protoperidinium conicum (Gran) Balech **Fig. 26**

[Palaeontological name: *Selenopemphix quanta* (Bradford) Matsuoka]

A brown smooth- walled cyst, covered with several rows of pointed spines. The cyst is compressed antero-posteriorly and is reniform in apical view. Cyst width 51-59μm, length 45-50μm and processes length 7-10μm. The hypocyst has two broad

antapical lobes. Viable cysts contained pink globular contents. None were successfully germinated.

The cyst is known from the Persian Gulf (Bradford & Wall 1984), however, it has not been reported from the Arabian Sea and the Gulf of Oman.

References: Bolch & Hallegraeff (1990), Fig.14; Sonneman & Hill (1997), Fig. 21; Joyce (2004b), Plate 7, Figs 1a-f.

Protoperidinium* cf. *thorianum (Paulsen) Balech **Fig. 27**

Synonym: *Peridinium thorianum* Paulsen, *Peridinium thorianum* Meunier

A large, dark brown, smooth-walled spherical cyst. Cyst diameter is 55μm. Archeopyle is subhexagonal and slightly convex on three sides and it is formed by the loss of an intercalary plate (1a or 2a).

Only one empty cyst was found in sediment. The cyst is larger in size compared with those described by Sonneman and Hill (1997) from Victorian/Australian coastal sediments. They found variation in the archeopyle shape of this type, however cyst can be distinguished by its larger size and darker colour from other species. *P.* cf. *thorianum* is also recorded from the Arabian Sea (Zonneveld 1997), but there is no morphological description or images in this study to compare with *P.* cf. *thorianum.*

References: Sonneman & Hill (1997), Fig. 16.

Protoperidinium* cf. *punctulatum (Paulsen) Balech **Fig. 28**

Synonym: *Peridinium punctulatum*

A spherical, smooth-walled brown cyst. Cyst diameter is 52μm. The archeopyle is subhexagonal with curved sides, reflecting the loss of the second intercalary plate. Only one specimen was found in sediments.

This cyst type differs from those described by Bolch and Hallegraeff (1990) by having a larger size and a different archeopyle shape. However, the archeopyle is almost similar in shape to the 2a intercalary plate with the motile cell of *P. punctulatum* which appeared in Wall and Dale (1968). This type has not been reported previously from adjacent tropical areas. Although the cyst of a gonyaulacoid species (*Bitectatodinum spongium* Zonneveld & Jurkschat) that was described from the Arabian Sea (Zonneveld & Jurkschat 1999, Plate II, Figs 2-3) possesses high similarity in the archeopyle shape and cyst size, but clearly differed from this cyst by its colour (yellow to colourless) and cyst wall ornamentation (spongy and granular-fibrous wall).

References: Wall & Dale (1968), Plate 3, Fig. 24; Bolch & Hallegraeff (1990), Fig. 18; Sonneman & Hill (1997), Fig. 29.

Protoperidinium leonis (Pavillard) Balech **Figs 29a-c**

Synonym: *Peridinium leonis* Pavillard

[Palaeontological name: *Quinquecuspis concretum* (Reid) Harland]

Pale-brown to dark brown cysts, pentagonal in outline. Cyst length 44-57μm and width 43-54μm. The cyst is dorso-ventrally compressed with a smooth or sometimes granular surface (Figs 29a-b).The epicyst is conical and may have shoulders (Figs 29a, c). The hypocyst forms two antapical horns with pointed tips (Fig. 29c) or may form two broad antapical lobes, the right one being bigger than the left (Figs 29a-b). The equatorial region is inflated and clearly defines the paracingular region (Fig. 29a). A triangular archeopyle is present on the epicyst and reflects the loss of 2a intercalary paraplates (Fig. 29c).

This cyst type was found at most sites, but none were germinated. Our specimens differ from those described for this species from recent Australian sediments by having a distinctly smaller size. A size range of 68-85μm long and 65-74μm long has been reported from that temperate areas by Bolch and Hallegraeff (1990) and Sonneman and Hill (1997) respectively. The size of 64μm long is also reported from

Gulf of California (subtropical region, Morquecho & Lechuga-deveze 2003). In addition, the smaller cyst size (40μm long) is known from the South China Sea where the area is affected by East Asian monsoon system (Kawamura 2004). The size of specimens from present study more closely resembles that from Mexican sediment (57μm long; Pena-Manjarrez *et al.* 2005). This type was reported in the Arabian Sea (Zonneveld 1997), but without any morphological details or photographs.

References: Bolch & Hallegraeff (1990), Fig. 20; Sonneman & Hill (1997), Figs 25c-d; Joyce (2004b), Plate 7, Fig. 4.

Protoperidinium minutum (Kofoid) Loeblich III **Fig. 30**

Synonym: *Peridinium minutum* Kofoid

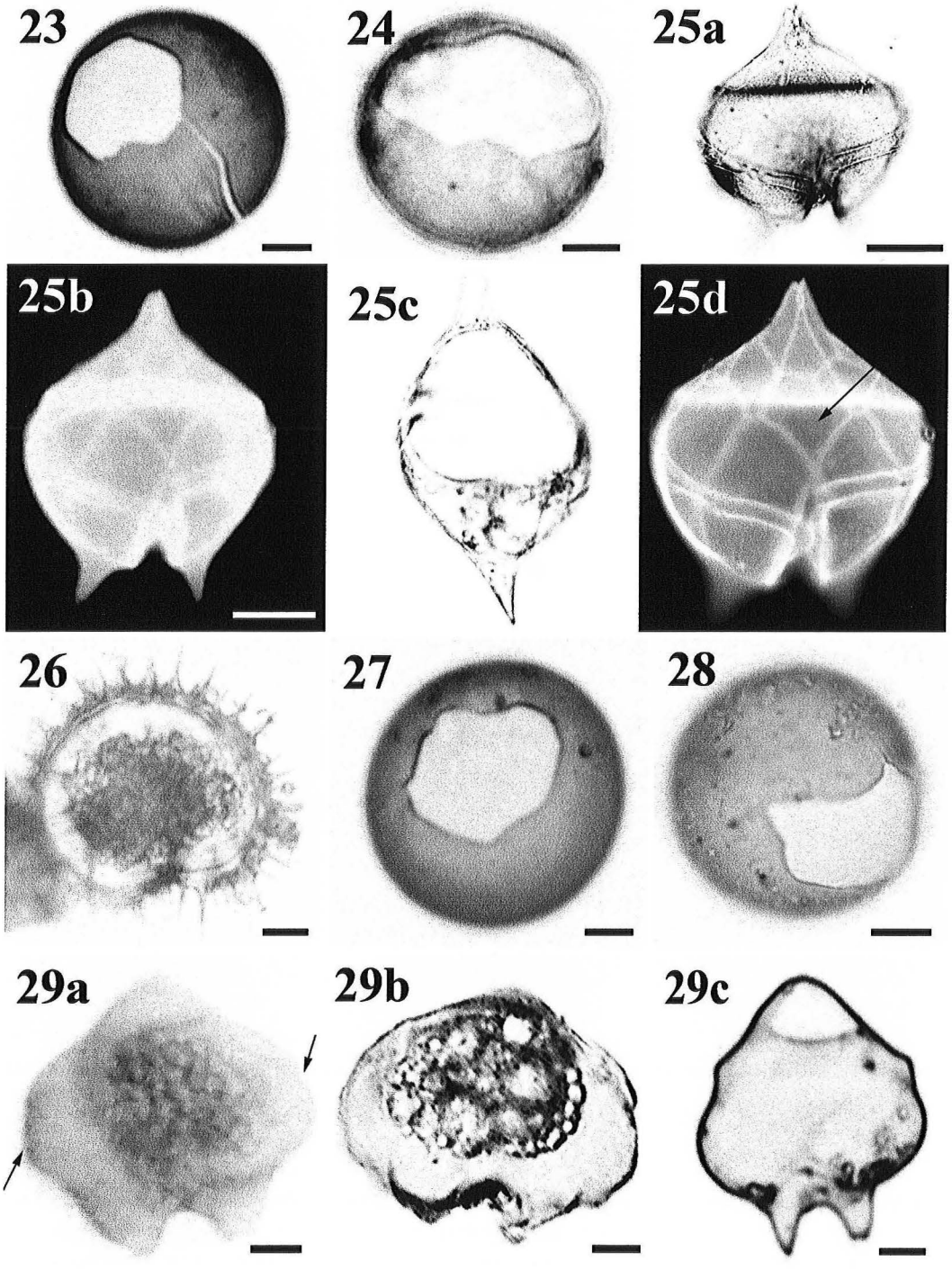
Spherical cysts, pale brown in colour covered with numerous short pointed spines. Cyst diameter is 27-29μm and spins length 8-13μm. The spines are straight or may be curved, spherical in cross-section and evenly distributed on the cyst (Fig. 30). The archeopyle was not observed in this study. This cyst type was not common and none were germinated.

This cyst type is differed from that described by Wall and Dale (1968) (Plate 4, Figs 6-7), and more resemble that described for the species by Bolch and Hallegraeff (1990) and Sonneman and Hill (1997). The cyst has not previously been reported from adjacent tropical areas.

References: Bolch & Hallegraeff (1990), Fig. 27; Sonneman & Hill (1997), Fig. 15.

Plate 7: *Protoperidinium* spp. resting cysts and thecate cells from southeast coast of Iran

Fig. 23. LM. Cyst of *Protoperidinium avellana* with a symmetrical archeopyle formed by loss of one intercalary plate.
Fig. 24. LM. Cyst of *Protoperidinium* cf. *denticulatum* with an archeopyle formed by loss of the two intercalary plates.
Fig. 25a. LM. Vegetative cell of *P. claudicans* isolated from mix-incubated sediment.
Fig. 25b. Dorsal view of Calcofluor-stained motile cell of *P. claudicans* isolated from mix-incubated sediment.
Fig. 25c. LM. Lateral view of theca cell of *P. claudicans* showing pointed antapical horn.
Fig. 25d. Ventral view of the same Calcofluor-stained cell exhibiting the four sided first apical plate (arrow).
Fig. 26. LM. *Protoperidinium conicum* cyst from an apical view showing pointed processes.
Fig. 27. LM. *Protoperidinium* cf. *thorianum* cyst showing the irregular archeopyle.
Fig. 28. LM. *Protoperidinium* cf. *punctulatum* cyst representing the archeopyle formed by loss of first intercalary plate.
Fig. 29a-c. LM. *Protoperidinium leonis.*
Fig. 29a. Cyst exhibits a distinctive paracingulum region (arrows)
Fig. 29b. Cyst with contents.
Fig. 29c. Empty cyst with a triangular archeopyle.
All scale bars=10μm.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Protoperidinium oblongum (Aurivillius) Parke & Dodge **Figs 31a-k**

Synonym: *Peridinium divergens* Ehrenberg var. oblonga Aurivillius.
[Palaeontological name: *Votadinium calvum* Reid]

The resting cysts are pentagonal and dorso-ventrally compressed. Cysts varied in size from 53-59μm in width and 50-63μm in length. These smooth-walled cysts possess a pale brown outer wall and showed considerable variation in morphology. Wall and Dale (1968) described three cyst types according to variations on their apical and antapical shape. Three types of cyst were also observed in Iranian sediment, one of which (Fig. 31a) is similar to those described by Wall and Dale (1968, Fig. 26) and Morquecho and Lechuga-Deveze (2003, Fig. 5). The first type has broadly rounded apical and antapical horns, with short antapical horns, giving the cyst a truncated appearance (Fig. 31a). In the second form, the epicyst has convex sides, whereas the hypocyst has straight sides and is divided into two distinct lobes (Fig. 31b). In the third type, the cysts are rhomboidal in outline with straight or slightly concave sides with symmetrical and triangular epicysts. The hypocyst also has straight sides tapering into two rounded distinct horns. The paracingulum is weakly defined (Figs 31c-k). Cysts show variety in cyst content and colour. In some cysts protoplasmic membrane is clear (Figs 31c, e, i & j); this appears in the cyst prior to excystment (Wall & Dale 1968). Cysts sometimes contain numerous pink oil globules (Figs 31d & f). Cysts may show convex sides on the epicysts (Fig 31j). The archeopyle was not observed as all specimens were intact. This third cyst form is different from *P. oblongum* described by Bolch and Hallegraeff (1990) and Sonneman and Hill (1997) due to its lack of paracingular grooves. It is more similar to those *P. oblongum* from South Africa (Joyce 2004b) and Russian Pacific coast (Orlova *et al.* 2004).

P. oblongum was very common in Iranian sediments. Two specimens (Figs 31c & 31e) were successfully germinated to produce colourless and elongate *Protoperidinium oblongum* cells (Figs 31g-h). The theca has two almost parallel elongate and conical antapical horns. The sulcus is set asymmetrically in the hypotheca; the right antapical horn is slightly displaced laterally from the sulcal

furrow. The motile cell also more closely resembles that described by Wall and Dale (1968). The cyst is also known from the Arabian Sea and southwest Indian recent sediments (Zonneveld 1997; Godhe *et al.* 2000). There are no morphological details of cyst or thecate cell from these areas for comparison.

References: Wall & Dale (1968), Plate 1, Figs 22-29; Bolch & Hallegraeff (1990), Fig. 19; Sonneman & Hill (1997), Fig. 26. Joyce (2004a), Fig. 18; Joyce (2004b), Plate 8, Figs 1a-f.

Protoperidinium pentagonum (Gran) Balech **Fig. 32a-b**

Synonym: *Peridinium pentagonum* Gran
[Palaeontological name: *Trinovantedinium capitum* Reid]

A colourless, pentagonal, peridinioid-shaped cyst, dorso-ventrally compressed. Cyst length 70-77μm, width 56-67μm. The cyst wall is decorated with frequent short, rigid and pointed spines. The epicyst tapers into an apical boss and the hypocyst carries two short antapical horns. Both the parasulcus and paracingulum are well developed and ornamented by rows of spines along their borders. The archeopyle was not observed in this study. None were successfully germinated.

Three morphotypes could be distinguished according to variation in antapical and epicystal regions. The first type (Fig. 32a) possesses a conical epicyst with convex sides, poorly defined antapical horns and rounded paracingulum region. The second type has a conical epicyst with a pointed apical horn, longer pointed antapical horns and well defined angular paracingular region (Figs 32b-c). The third type has a clearly rounded equatorial region, and well defined apical and antapical horns extending to short spines. Only the third type was surrounded by a mucilaginous layer with adherent detritus particles (Figs 32d-f). The first and second types are similar to previously described cysts of this species (e.g. Bradford & Wall 1984; Bolch & Hallegraeff 1990; Joyce 2004b; Joyce *et al.* 2005), but the third type is distinct from them and has not previously been documented.

Cysts of *P. pentagonum* were common in the sediments. *P. pentagonum* is also known from the Persian Gulf, the Gulf of Oman, the Arabian Sea and southwest India (Bradford & Wall 1984; Godhe *et al.* 2000).

References: Bolch & Hallegraeff (1990), Fig. 15; Orlova *et al.* (2004), Figs 56-60; Joyce (2004a), Fig. 19; Joyce *et al.* (2005), Fig. 16.

Protoperidinium* cf. *conicoides (Paulsen) Balech **Fig. 33**
[Palaeontological name: *Brigantedinium simplex* (Wall) Reid]

A brown, spherical, smooth-walled cyst. Cyst diameter 49μm. The archeopyle is trapezoidal slightly curved in two parallel sides. The shape of the archeopyle represents loss of the second intercalary paraplate.

This cyst type is slightly larger in size than those (35-40μm) described by Bolch and Hallegraeff (1990) from Tasmanian/Australia sediments. The species generally is cold water species (Bolch & Hallegraeff 1990), however, cysts have been found within a broad range of temperature (-2.1˚C -29.6˚C) (Marret & Zonneveld 2003). Marret and Zonneveld (2003) reported *Brigantedinium* spp. including *B. simplex* from the Arabian Sea and the Gulf of Oman, however, there is no information on the cyst size and morphology from these tropical areas.

Reference: Wall & Dale (1968) Plate 4, Fig. 29; Bolch & Hallegraeff (1990), Fig. 26; Fensome *et al.* (1993), Fig. 142 H.

***Protoperidinium* subinerme** Paulsen **Fig. 34**
[Palaeontological name: *Selenopemphix nephroides* Benedek]

Pale-brown smooth-walled cyst with a deeply excavated paracingulum. The cyst diameter 42-45μm. In apical view, the cyst is ventrally concave and appears to be reniform to ovoid (Fig. 34). The cysts possess a conical epicyst with concave sides extending into an apical horn. The hypocyst is tapered into the dorso-ventrally

flattened ridge. The hexagonal intercalary archeopyle was not observed. None had viable contents; therefore germination experiments were not carried out.

This cyst type was not a common type in the sediment. The cyst closely resembles in size to those documented from the Persian Gulf and the Gulf of Oman (Bradford & Wall 1984) and Russian Pacific coast (Orlova *et al.* 2004), but differs in size from that described from south and east China Sea (30-35μm wide, Qi *et al.* 1996). It is also known from the Persian Gulf, the Gulf of Oman and the Arabian Sea, southwest India, and the Somalian upwelling area (Bradford & Wall 1984; Zonneveld & Brummer 2000, Godhe *et al.* 2000; Marret & Zonneveld 2003).

References: Bolch & Hallegraeff (1990), Fig. 16; Sonneman & Hill (1997), Fig. 30; Orlova *et al.* (2004), Fig. 61.

***Protoperidinium* sp. 1** **Figs 35a-e**

A dark brown, pentagonal, dorso-ventrally compressed cyst with a smooth wall. Cyst length 70μm, width 65μm. The cyst has a red accumulation body and globular contents (Fig. 35a).The epicyst has a large triangular archeopyle that may reflect the loss of intercalary and apical paraplates. The archeopyle exhibits a distinct flattened hump on its anterior side and the operculum may remain attached to this part (Figs 35d-e). The hypocyst is straight-sided and forms two antapical lobes.

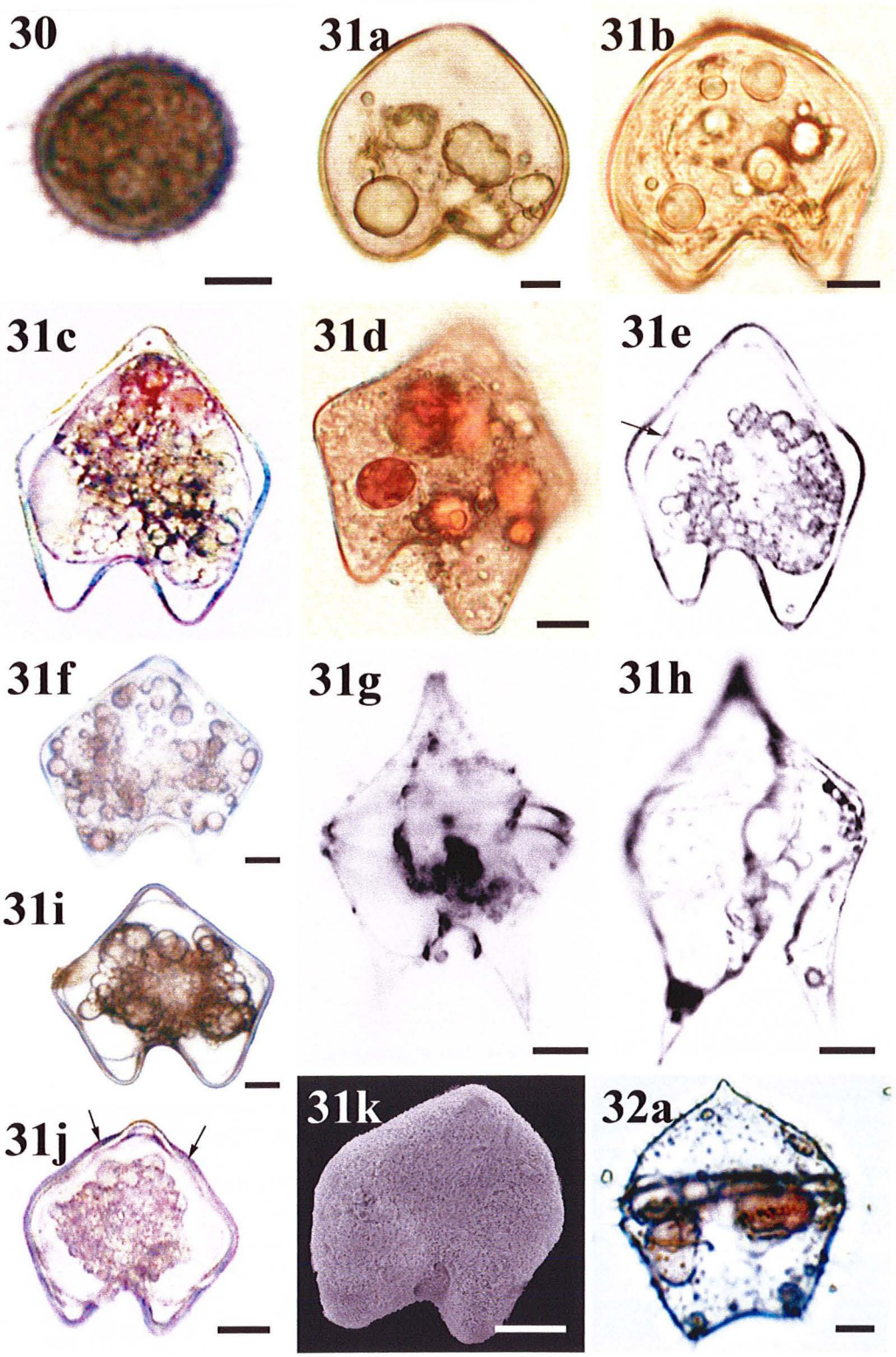
This cyst was successfully germinated to produce a colourless peridinioid cell with a convex conical epitheca extending into a conical apical horn (Figs 35b-c). The hypotheca is characterised by two conical antapical horns that diverge distally forming an angle of 120° between them and approximately 90° between the horns and the posterio-lateral body edge of the main body.

This cyst can be easily confused with cysts of *P. oblongum*. However, *Protoperidinium* sp. 1 has a distinct archeopyle with a flattened hump on the anterior edge. The motile cell morphology is also quite distinct due to diverging antapical

horns. We have been unable to find descriptions of *Protoperidinium* species with similar antapical morphology, therefore the motile cell remains unidentified.

Plate 8: *Protoperidinium* spp. resting cysts and theca cells from southeast coast of Iran

Fig. 30. LM. *Protoperidinium minutum* cyst with dense pointed processes.
Figs 31a-j. LM. *Protoperidinium oblongum.*
Fig. 31a. Cyst type 1 with two short antapical lobes.
Fig. 31b. Cyst type 2 showing the straight sides in hypocyst.
Fig. 31c. Cyst type 3 showing conical epitheca with straight sides.
Fig. 31d. Cyst showing large orange oil droplets.
Fig. 31e. Cyst showing clear protoplasmic membrane (arrow).
Fig. 31f. Cyst showing lots of pink oil droplets.
Figs 31g-h. Ventral view of the germinated cells showing an elongate pentagonal body.
Fig. 31i. Cyst showing brown colour.
Fig. 31j. Cyst with slightly convex epicyst sides (arrows).
Fig. 31k. SEM. Cyst of *P. oblongum.*
Fig. 32a-f. LM. *Protoperidinium pentagonum.*
Fig. 32a. Cyst type 1 showing convex epicystal sides. Note poorly defined antapical horn.
All scale bars =10μm.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31a
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31h
31i
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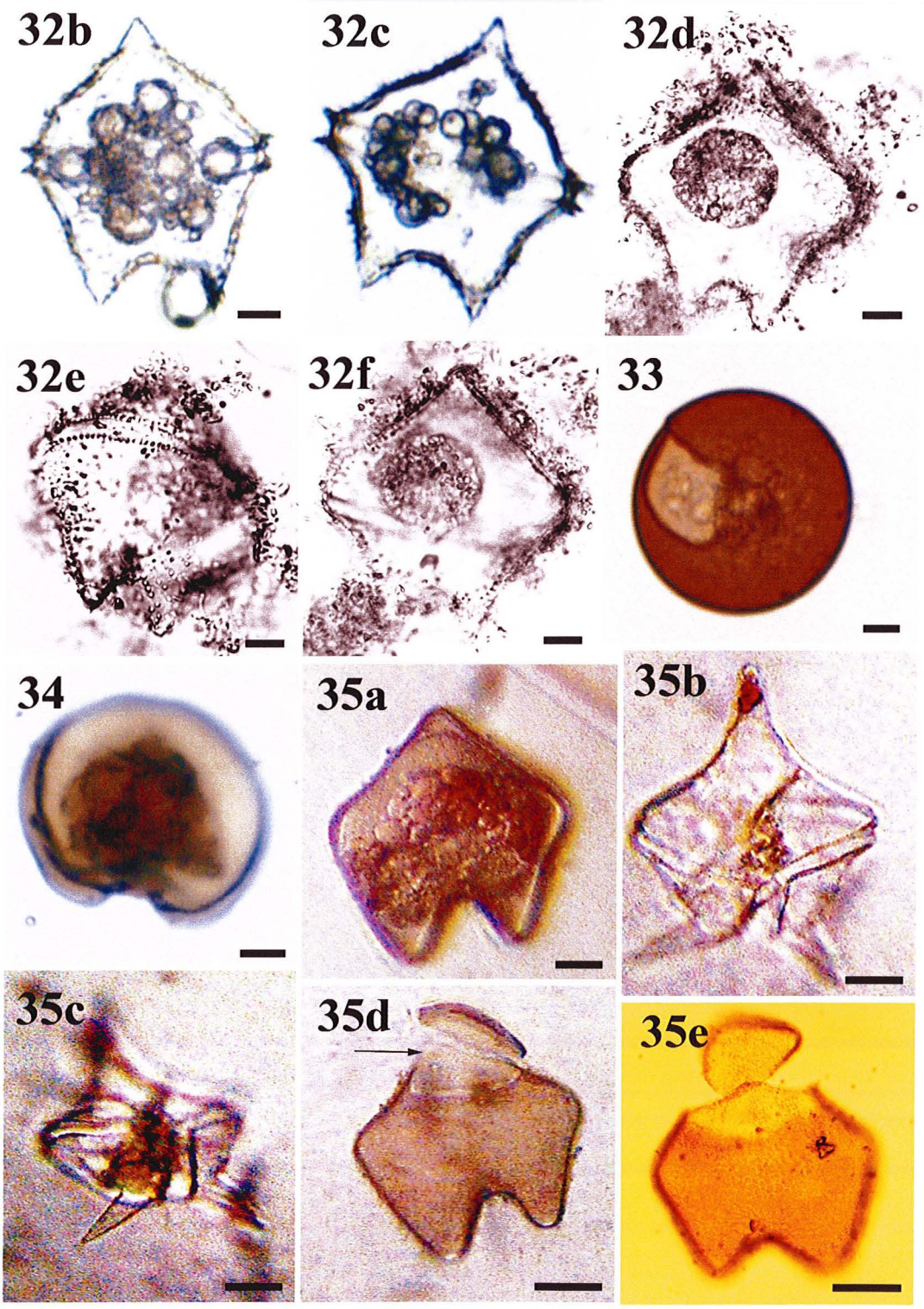
32a

Plate 9: *Protoperidinium* spp. resting cysts from southeast coast of Iran

Fig. 32b. LM. *Protoperidinium pentagonum* cyst type 2 showing straight epicystal and hypocystal sides.
Figs 32d-f. *Protoperidinium pentagonum* cyst type 3 with adherent mucilaginous particles. Note the curved paracingular region.
Fig. 33. LM. *Protoperidinium* cf. *conicoides* cyst showing a typical trapezoidal archeopyle.
Fig. 34. LM. *Protoperidinium subinerme*. Live cyst in apical view.
Figs 35a-e. LM. *Protoperidinium* sp. 1.

Fig. 35a. Live cyst with globular contents.
Figs 35b-c. Motile cell germinated from the cyst in Fig. 35a. Note diverging antapical horns.
Figs 35d-e. Empty cyst showing archeopyle with attached operculum. Note the broad hump with a flattened top on ventral side of the archeopyle (arrows).

All scale bars =10μm.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***Protoperidinium* sp. 2** **Fig. 36**

Pale brown, smooth walled pentagonal cyst that is dorso-ventrally compressed. Cyst length is 62-64μm, width 45-49μm. The cyst has a conical epicyst with convex sides tapering into an elongated apical horn. In outline the hypocyst is slightly concave and extended to two unequal antapical horns, of which the left is larger. None were successfully germinated.

***Protoperidinium* sp. 3** **Fig. 37**

Dorso-ventrally compressed, pale brown, pear-shaped cyst with a smooth wall. The cyst length is 62μm, width 47μm cyst. The conical epicyst is slightly curved at the sides and merging distally with the conical apical horn with a weakly developed sulcal region. The cyst is characterised by its two distinctly short antapical horns. The epicyst is longer than the hypocyst. The paracingular region is poorly defined and broadly rounded. Only one specimen was found in sediment, but it was not successfully germinated.

***Protoperidinium* sp. 4** **Fig. 38**

Pentagonal, brown-walled cyst with a smooth cyst wall. Cyst length is 45-48μm, width 55-59μm. The epicyst has concave sides, and tapers to a round apical horn. The hypocyst is excavated and bears two short lobes. The paracingular region is defined by two broad arms, which have relatively straight edges. This cyst is similar to *P. subinerme* but can be differentiated by the lack of a deeply excavated cingulum. This cyst type was not a common cyst and none was germinated.

***Protoperidinium* sp. 5** **Fig. 39**

Large, pentagonal, dark brown cysts with a smooth wall. Cyst length is 69μm, width 6μm. The epicyst has prominent concave sides, and the apical region is rounded.

This cyst is similar to *P. leonis* and *P. oblongum* but is larger in size and has a convex epicyst. Only two specimens were found and germination remained unsuccessful.

***Protoperidinium* sp. 6** **Fig. 40**

A rectangular, brown cyst with a smooth surface and round sides. Cyst length is 47μm, and width is 67μm. The epicyst is characterised by lack of apical and antapical horns, therefore in ventral view it shows a flat apex and convex sides. The hypocyst bears two broad, round antapical lobes. Only one specimen, without viable contents, was found during the quantitative survey.

***Protoperidinium* sp. 7** **Fig. 41**

Dark brown, round cysts with smooth wall surface. Cyst diameter is 40-44μm. The cyst contents are largely starch and lipid granules. The cyst has two thickly layered walls, where the outer layer is dark brown and the inner layer possesses a lighter colour. Sometime the outer layer is surrounded by a colourless membranous layer. All specimens were intact, therefore the archeopyle was not observed. None were successfully germinated.

These cysts were the most common peridinioid cyst type found in Iranian sediments. However, without germination studies or being able to examine the archeopyle, identification is not possible. This group may include several peridinioid species that form brown-round resting cysts in this size range.

***Protoperidinium* sp. 8** **Figs 42a-b**

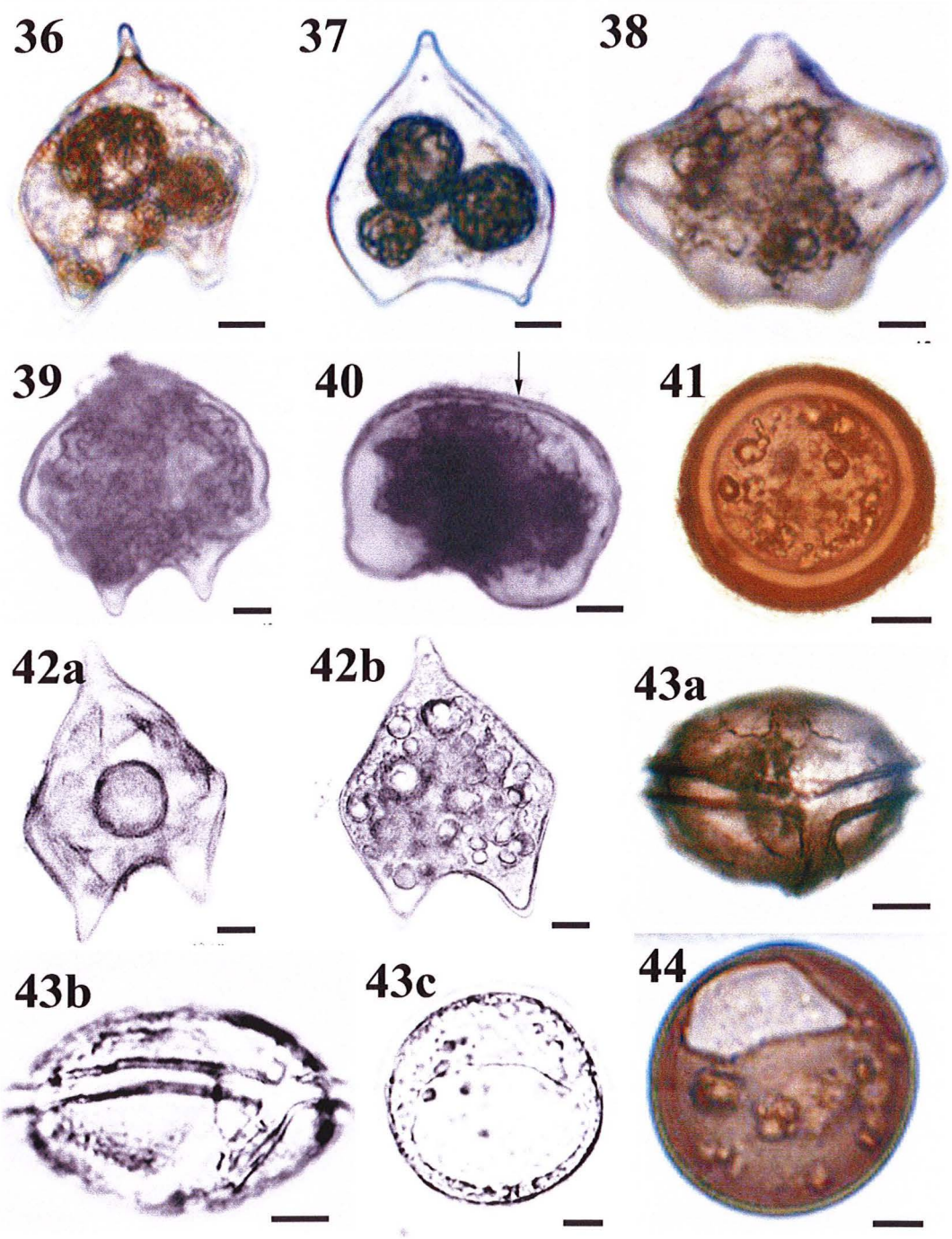
Pentagonal, smooth-walled purple-brown cysts dorso-ventrally compressed. Cyst length 72-78μm, width 50-56μm. This cyst is characterised by its elongate apical horn. The epicyst is conical with straight sides, tapering to an elongated conical horn.

plates are involved. The archeopyle resembles that of *Diplopelta parva* (Abe) Matsuoka by Fensome *et al.* (1993). However, *D. parva* possess a spiny cyst wall and are smaller in size (27-30μm) (Bolch & Hallegraeff 1990).

Reference: Fensome *et al.* (1993), Fig. 145.

Plate 10: *Protoperidinium* spp. and *Diplosalis* spp. resting cysts and thecae from southeast coast of Iran

Fig. 36. LM. *Protoperidinium* sp. 2 cyst showing an elongate apical horn. Note the convex epicyst.
Fig. 37. LM. *Protoperidinium* sp. 3 cyst with short antapical horns.
Fig. 38. LM. *Protoperidinium* sp. 4. Note the weakly defined paracingulum region.
Fig. 39. LM. *Protoperidinium* sp. 5 cyst.
Fig. 40. LM. *Protoperidinium* sp. 6 cyst showing flattened apex (arrow)
Fig. 41. LM. *Protoperidinium* sp. 7. Round brown cysts with darker colour of outer wall layer.
Fig. 42. LM. *Protoperidinium* sp. 7. Large cyst with elongated apical horn.
Fig. 43a-c. LM. *Zygabikodinium lenticulatum.*
Fig. 43a. Ventral view of cyst. Note paracingulum and parasulcus.
Fig. 43b. Vegetative cell in ventral view showing the cingulum and sulcus.
Fig. 43c. Vegetative cell in apical view.
Fig. 44. LM. *Diplopsalis* sp. cyst showing subtrapezoidal archeopyle.
All scale bars =10μm.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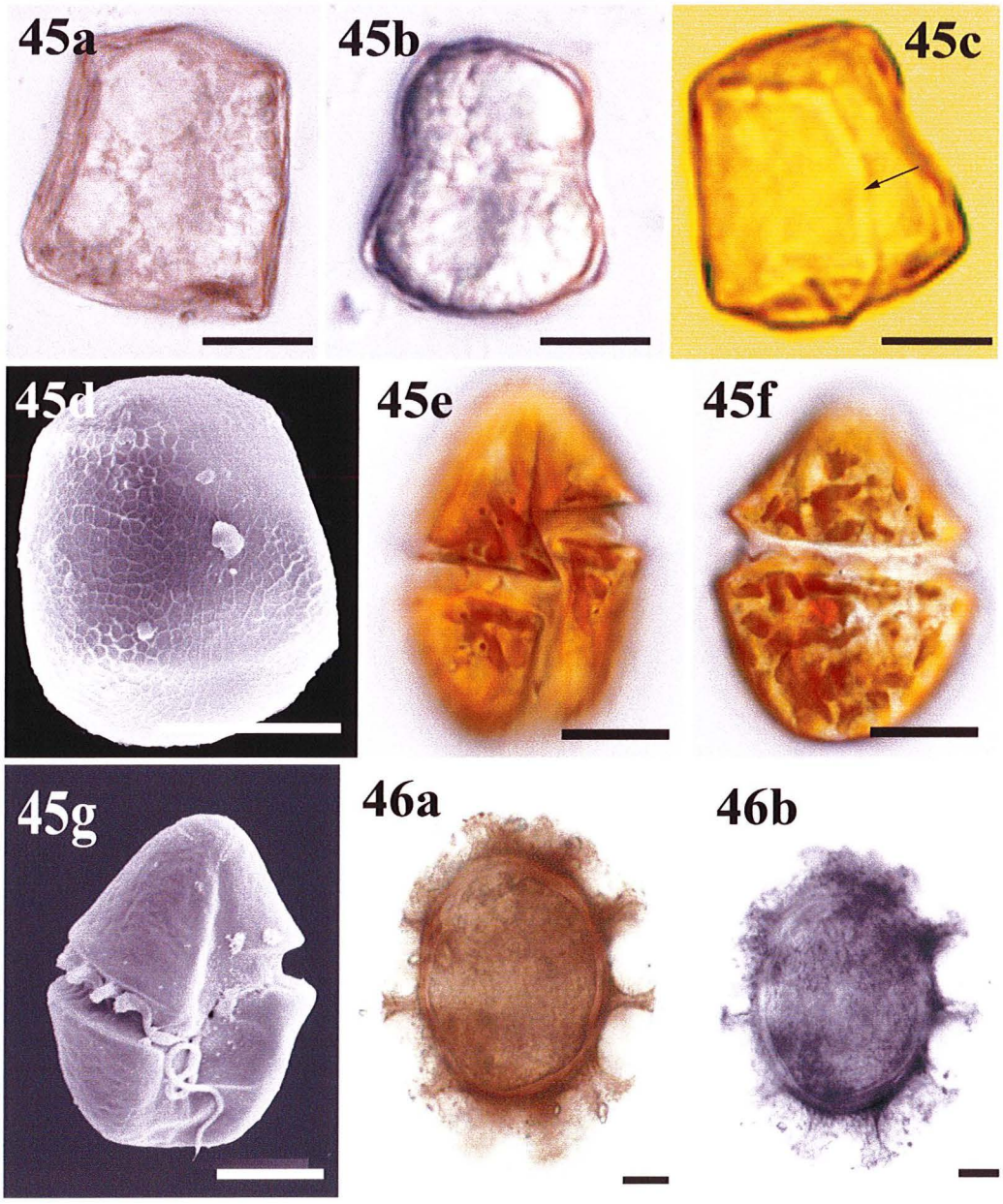
Order: Gymnodiniales

Family Gymnodiniaceae (Bergh) Lankester.

Genus: ***Gymnodinium*** Stein

***Gymnodinium* sp.** **Figs 45a-g**

A pale brownish-purple small cyst with a trapezoidal to sub-rectangular outline. Cyst length is 23-34μm, and width 17-27μm. The cyst is convex on two parallel longer sides and slightly concave on the two shorter sides (Figs 45a, b & d). Cysts have dark globular contents (Fig. 45a). The cyst wall possesses a reticulate surface structure that reflects the pattern of amphiesmal vesicles of the motile cell. Features that are reflected within network structure include the paracingulum and parasulcus (Fig. 45d). A chasmic archeopyle is present (Fig. 45c). This was a common cyst type in south coast Iranian sediments. Two cysts were successfully germinated to produce a pale golden gymnodinioid cell. Examinations of germinated specimens suggest that the archeopyle is chasmic and oriented along the line of the sulcus. The motile cell's epitheca is conical with a rounded apex, and the hypotheca is rounded or sometimes truncated. The epitheca is smaller than hypotheca in size (see Chapter 3 for more details on cyst and motile cell morphology). This cyst form has not previously been reported from either recent or fossil sediments. Morphological and molecular analyses indicate that this dinoflagellate is related to other micro-reticulate cyst-forming species, *G. catenatum*, *G. nolleri*, and *G. microreticulatum* (see Chapter 3).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Family Polykrikaceae Kofoid & Swezy

Genus: ***Polykrikos*** Butschli

Polykrikos kofoidii Chatton **Figs 46a-b**

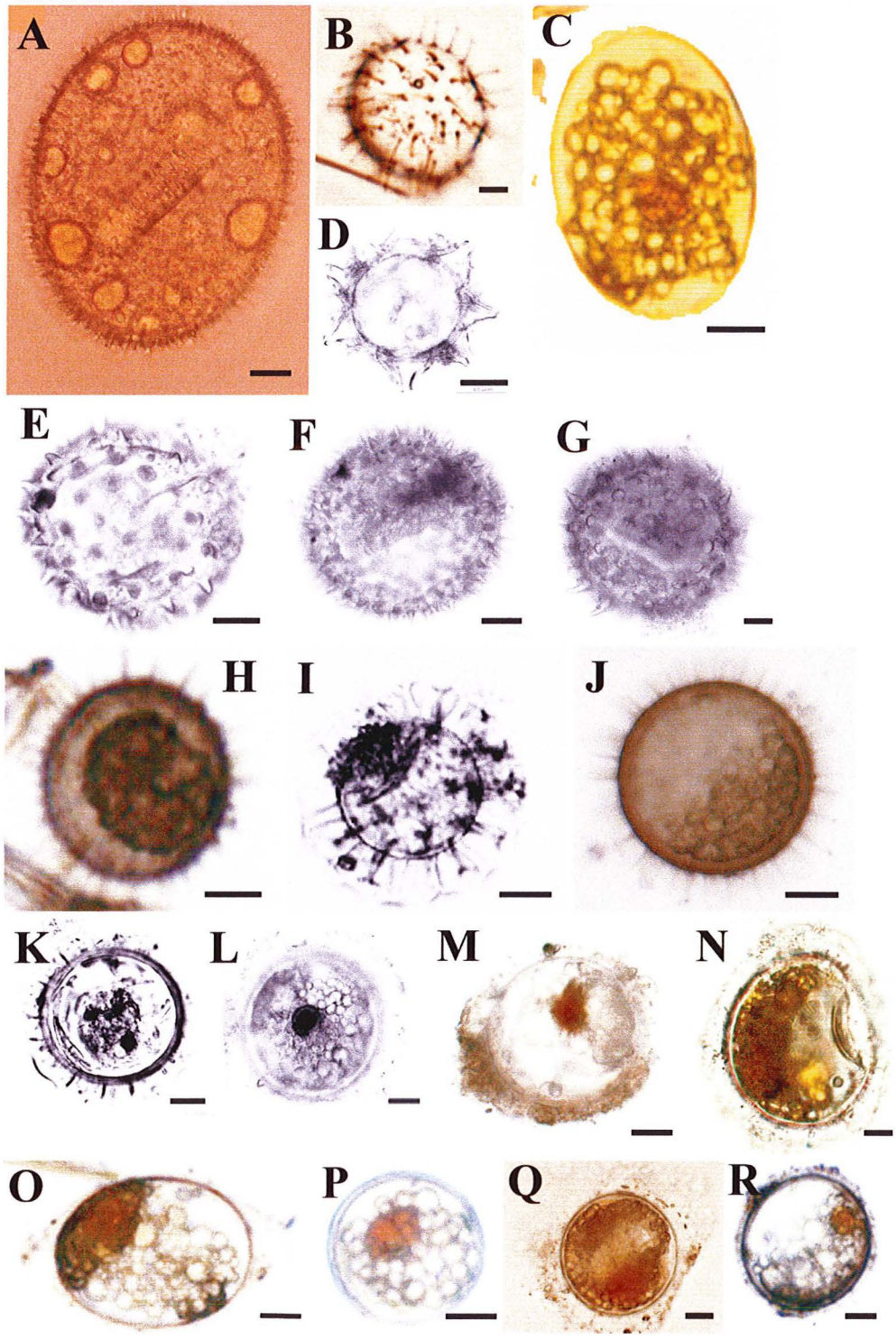
A pale brown elongate cyst is covered by numerous fibrous flared processes. The cyst length is 52-57μm and width 46-49μm. The processes are hollow and short, cylindrical in shape, and distally open. The tremic archeopyle (Matsuoka 1985) has a circular shape, and is formed in the apical region. This was not a common cyst type in the samples; only three cysts were found and none were successfully germinated.

The cyst is also documented from the Arabian Sea (Zonneveld 1997; Zonneveld *et al.* 2001).

References: Haifeng *et al.* (2003), Plate III, Fig. 27; Dongzhao *et al.* (2003), Fig. 14; Joyce (2004b), Plate 4, Fig. 1.

Plate 12: Unidentified putative resting cysts from southeast coast of Iran

Fig. A. A large cyst showing short, pointed, and evenly distributed spines. Note cysts content including globular contents and oil droplets with no obvious accumulation body.
Fig. B. Spiny clear cyst with capitate spines.
Fig. C. Oval cyst showing globular contents with orange accumulation body.
Fig. D. Spherical cyst showing sub-conical processes in cross-section.
Fig. E. Spherical cyst surrounded with numerous short and pointed processes.
Fig. F. Cyst showing short and pointed processes. Cyst is differed from the cyst type E by its green colour.
Fig. G. Cyst showing short conical or curved processes that emerge from wider base. Cyst after hydrochloric acid treatment, note spines still present.
Fig. H. Dark wall cyst with long pointed processes showing the contents.
Fig. I. Cyst surrounded with evenly distributed process with bifid tips.
Fig. J. Cyst with clear brown outer wall showing pointed and relatively long process. Note cyst colour is differed from cyst type H.
Fig. K. Spherical cyst showing short cylindrical processes that surrounded with a thin outer membranous layer.
Fig. L. Spherical, smooth-walled cyst surrounded with the mucilaginous layer and showing a dark red accumulation body and globular contents.
Fig. M. Spherical, green, smooth-walled cyst enclosed in mucilaginous layer and sediment particles. Note, cyst differed from cyst type L by colour and size.
Fig. N. Ovoid smooth-walled cyst surrounded with mucilaginous layer showing a yellow accumulation body and globular contents.
Fig. O. Oval cyst showing oil droplet content and an orange accumulation body. Note the mucilaginous layer around the cyst.
Fig. P. Small, smooth-walled, spherical cyst showing lipid globules and a red accumulation body.
Fig. Q. Spherical, pale brown cyst enclosed in mucilaginous layer, with a granular contents and an orange accumulation body.
Fig. R. Spherical smooth-walled cyst showing clear dark colour in outer wall, globular contents and yellow accumulation body.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4. Discussion

4.1. Dinoflagellate Cyst Composition

Cyst abundance and diversity varied greatly between locations (Table 3). Cysts were more abundant in Pasabandar coastal sediments compared to the two other locations (Chabahar Bay and Bahoo-Kalat estuary). The abundance of the cyst assemblage at Pasabandar may be explained by two important factors. The highest dinoflagellate cyst concentrations are usually found in muddy, rather than sandy sediment (Dale 1983; Nehring 1994). The influence of sedimentology on the concentration and abundance of fauna and cyst flora has been documented by many authors. Wang *et al.* (2004) recorded higher dinoflagellate cyst assemblage in fine muddy sediments. Joyce *et al.* (2005) also found higher dinoflagellate cyst concentration in muddy sediment. Bradford and Wall (1984) also found that the sediments of the Gulf of Oman varied between coarse sand and mud, with sandy sediment showing only a very limited cyst flora. Dinoflagellate cyst concentrations also appear to correspond well with the sediment type; the highest cyst concentrations were recorded in Pasabandar which has a fine muddy sediment and lower concentrations were recorded in Bahoo-Kalat estuary which has sandy-silt sediment. Sites in Chabahar Bay, with a clay- silt sediment regime, possessed a higher cyst concentration than Bahoo-Kalat sites.

In addition, the other factor that may influence cyst assemblage is the abundance of vegetative cells. The vegetative cells of dinoflagellates are reported to be rare in Bahoo-Kalat estuary where the dominant group of phytoplanktons are diatoms (Khodami 2004). The Pasabandar coastal area is associated with occurrence of dinoflagellate blooms, but there is no information on the dinoflagellate species from this area. Bradford and Wall (1984) found a high abundance of *Protoperidinium* cysts dominated by *P. pentagonum* in the open marine bays of the Persian Gulf. High relative abundance of the heterotrophic *Protoperidinium* cysts is found in areas enriched with nutrients and with high primary production (Dale 1996; Wang *et al.* 2004). Haghighy *et al.* (1996) found high primary productivity in Chabahar Bay.

Therefore, the high relative abundance of *Protoperidinium* cyst in Chabahar Bay could be the result of high primary productivity of the bay.

4.2. Comparisons with Adjacent Tropical Areas

The dinoflagellate cyst assemblage found in this study differs from those of previous studies of tropical regions of the Persian Gulf and the Arabian Sea that used palynological acid preparation methods (e.g. Bradford & Wall 1984; Zonneveld 1997) that destroy calcareous cyst types. Bradford and Wall (1984) reported 31 species of organic-walled dinoflagellates from recent sediment in the shallow water of the Persian Gulf (Iranian border), of which *Pyrodinium bahamense* (=*Polysphaeridium zohari* Rossignol) was the dominant species. *Pyrodinium bahamense* is a causative species of PSP in many tropical areas, however, it was not found in this study. *Pyrodinium* cysts have also been reported from Kuwait sediments (i.e. see Fig. 1 for geographical location of Kuwait in the extreme west of the Persian Gulf) by Husain and Lewis (2004). Moreover, the cyst is known from other tropical areas such as Manila Bay in Philippines (Azanza *et al.* 2004). *P. bahamense, L. polyedrum* and *Pyrophacus steinii* are considered to be typical neritic cyst flora of the warm waters found in the Persian Gulf (Bradford & Wall 1984). Of these, the last two species are present in Iranian sediments. Bradford and Wall (1984) pointed out that the abundance of *Pyrodinium* cysts decreased from the western to the eastern regions of the Persian Gulf. Although its cyst was present in the Gulf of Oman in low numbers, its motile cell has not been reported.

The motile cell of *P. bahamense* is thought to be restricted to warm water areas (Wall *et al.* 1977). It can be found for all or part of the year in tropical waters with high salinity (up to 35.7%) close to mangrove swamps with little water exchange with the open ocean (Wall & Dale 1969). The Persian Gulf has a neritic, highly saline (more than 40% salinity) environment, with a counter-clockwise water circulation pattern, which differs from the open marine environment of the Gulf of Oman and the Arabian Sea (Bradford & Wall 1984). Some estuaries and bays on the southeast coast of Iran are also surrounded by mangrove trees (e.g. Gwatar Bay and Bahoo-Kalat estuary). Bahoo-Kalat's location toward the end of the estuary is

surrounded by mangrove trees (*Avicenna marina)*. However, from the middle towards the entrance of the estuary (our sampling site), these trees are totally absent (Zarei 1993). Although the areas in the present study have salinity (37.5-35.9 %) that is favourable for the growth of *P. bahamense*, the site does not have mangrove trees. In addition, the circulation of water in the Gulf of Oman is strongly affected by seasonal monsoon circulation (Bradford & Wall 1984) that could influence the presence of the cyst in the area.

Protoperidinium species were the dominant cysts in the Arabian Sea and more than 80% abundance was reported from upwelling regions along the Yemen, Oman, and Pakistan coasts (Zonneveld 1997). Distribution of most dinoflagellate cysts in the Arabian Sea and the Gulf of Oman is highly affected by monsoon currents; for example, the relative abundance of *P. bahamense* cysts was found to be related to the southwest monsoon, and its highest relative abundance was in Yemen and the Oman shelf, but this species composed less than 1% of the cysts form the Arabian Sea (Zonneveld 1997). High cyst concentrations of *P. bahamense* were found during southwest monsoon in manila bay off Philippine (Azanza *et al.* 2004). However, some species such as *L. polyedrum, Spiniferites* spp., *P. pentagonum* and *P. Leonis* show no clear relationship with southwest (SW) or northeast (NE) monsoon current (Zonneveld 1997). It appears that species which have no correlation with monsoon current are more common in Iranian sediment. Our sampling was taken during the NE monsoon. Previous studies (e.g. Nikouyan *et al.* 1998; Attaran-Fariman 2000; 2001; 2002, Haghighy *et al.* 1996) on benthic fauna and phytoplankton flora in Iranian southeast coastal waters suggested that the SW monsoon has strongly affected the distribution diversity and assemblage of fauna and flora, whereas the NE monsoon current has less effect on the assemblage of the fauna and flora. However, Khodami (2004) documented some species of dinoflagellates where vegetative cells are present at one site in the Bahoo-Kalat estuary, only after the NE monsoon (e.g. *Gymnodinium* sp.). All these studies show that diversity, abundance and assemblage of fauna and flora are affected by monsoon currents in Iranian waters. These examples may explain the lack of some typical warm water species such as *P. bahamense* from this area. Therefore, a specific survey examining the effect of monsoon current on the assemblage of dinoflagellate cysts should be considered in this region.

Among *Protoperidinium* and gonyaulacoid cysts found in this study, several other species were also common in the Persian Gulf, such as: *Spiniferites bentori S. bulloideus, S. membranaceus S. mirabilis* and *P. pentagonum*. Husain and Lewis (2004) investigated dinoflagellate cysts from Kuwait sediment and identified 16 species belonging to *Scrippsiella, Protoperidinium, Gonyaulax*, and *Pyrophacus* genera. Wall and Dale (1968) describe five fossil and sub-fossil cysts from the western Arabian Sea, of which only *Protoperidinium pentagonum* is similar to the first type of this species in the present study. Wall and Dale (1968) in another work, investigate calcareous dinoflagellates in Quaternary marine sediment of some areas, including western Indian Ocean. They reported only *Scrippsiella sweenyae* (Balech) Loeblich from the western Arabian Sea, which is more or less similar to *Scrippsiella* sp. 2 from present study (see Chapter 4).

Marret and Zonneveld (2003), from their work on the distribution of dinoflagellates in recent sediment on a global scale, used palynological methods and reported several species from the Arabian Sea. Of those species, *Lingulodinium polyedrum, Spiniferites mirabilis, Protoceratium reticulatum, Protoperidinium conicum, P. denticulatum, P. avellana, P. subinerme and Pyrophacus steinii* are analogous to those of the present study. *Pyrophacus steinii* are found in the present study occur only in very low numbers. The cyst was also recorded from the Gulf of Oman and the northern Arabian Sea in low numbers (Bradford & Wall 1984). In addition, Wall *et al.* (1977) documented that this species is characterised as a tropical or warm water species that often occurs in low numbers. There is no other information on cyst assemblages in the Gulf of Oman or the northern part of the Arabian Sea where cysts have not been prepared with hydrochloric acid that destroys calcareous cyst types and can distort the dinoflagellate cyst assemblage.

The cyst assemblage of this study more closely resembles that described in south-western India by Godhe *et al.* (2000), using similar preparation methods to those used in the present study. In that tropical region both *Pyrophacus steinii* and *P. bahamense* (which are considered as warm water species) were absent, and also the most abundant species in estuary sites was *Scrippsiella trochoidea.* Moreover, the Iranian dinocyst assemblage also resembles that of other subtropical and equatorial

areas (e.g. Morquecho & Lechuga-Deveze 2003, Joyce 2004b), except for the total absence of some genera such as *Nematosphaeropsis* found in South Africa. Some cyst species in Iranian sediment are comparable to those of southern hemisphere species; for example in Tasmania (Bolch & Hallegraeff 1990) and Victoria (Sonneman & Hill 1997). In spite of this, cyst composition in Iranian sediments is different from that of Tasmanian sediments, as Gonyaulacoids dominate the cyst flora in these temperate areas.

4.3. Cysts of Toxic Dinoflagellates

Mapping of dinoflagellate cysts in coastal sediments can provide valuable information on the distribution of potentially harmful species in an area where blooms have occurred, or where vegetative growth may occur in future (Matsouka 1999, Bolch & Hallegraeff 1990). Despite the dominance of *Scrippsiella* spp. in the sediment of Iran's southeast coast, 2 potentially toxic cyst species were found in the sediment: *Alexandrium* cf. *tamarense* and *Lingulodinium polyedrum*.

A. tamarense is known as a major Paralytic Shellfish Poisoning (PSP) causative dinoflagellate (Taylor & Fukuyo 1998). *Alexandrium* cf. *tamarense* was found only in the Pasabandar coast where it was not a common species. However, its toxicity is unknown as the cyst did not germinate. The detection of cysts of *Lingulodinium polyedrum* is also interesting in the Pasabandar regions. This species is a potentially toxic species that produce yessotoxins (YTX) (Paz *et al.* 2004). The toxic syndrome of YTX is not well-defined in humans, but is linked with the mortality of fish and other marine organism (Joyce 2004b). *L. polyedrum* was the most common species among gonyaulacoid species in Iranian sediment. This species was found along the Pasabandar coast and in Chabahar Bay. Along the southeast coast of Iran, red tides occur regularly during the year especially after the SW monsoon, but the most common place for red tide is the Pasabandar coast (Khodami 2005 pers. com.). In November 2001 in Pasabandar (after the SW monsoon) blooms of *Gymnodinium* sp. and *Gonyaulax* sp. killed 15 tons of marine organisms (Shakoory 2004) and caused considerable economic loss. Blooms of *Chattonella* sp. and *Protoceratium* sp.

in October 2003 from this area has also been reported to have caused high mortality of the fish *Otolithes cuvieri* (Shakoory 2004).

Dinoflagellate cysts act as silt-like particles in the water column and can easily be moved by water currents (Anderson & Lively 1985) such as monsoon current in Arabian Sea. This may explain the absence of *Protoceratium* species in the sediments despite regular blooms of *Protoceratium* in the area. Despite the fact that Harmful Algal Blooms (HABs) are a serious problem in this area, no survey has been done to identify phytoplankton at species level. Most information and reports on red tide from this area only reveal the genus of the species. Therefore, the presence of the toxic cysts from this study can not be linked to the presence of the red tides.

In addition to the above-mentioned toxic species, *Scrippsiella trochoidea* can be a potentially harmful species. This species is not a toxic species to humans but it can cause fish kills (Hallegraeff & Bolch 1992; Matsuoka & Fukuyo 2000). It has been reported that dense concentration of this species caused red tide and associated fish mortality by producing anoxic conditions (Hallegraeff & Bolch 1992). This species is widespread along the Iranian coast. Cysts of *Gymnodinium catenatum* and cyst of *P. bahamense*, another two causative PSP were not found in this survey. Although a microreticulate cyst of *Gymnodinium* sp. was the second most common species in two locations, this species appears to be non-toxic (see Chapter 3).

5. Conclusion

This study provides the first study of the dinoflagellate cyst flora of the tropical region of southeast Iran. Dinoflagellate resting cysts were found in all of the sediment samples collected from an estuary (Bahoo-Kalat estuary) a coastal area (Pasabandar) and a Bay (Chabahar Bay). Although cysts were found in all sediments with different grain size, the cyst abundance was highest in muddy sediments and lowest in sandy-silt sediments. The cyst assemblage of the Iran southeast coast differs markedly from that of the southwest coast (Persian Gulf) as described by Bradford and Wall (1984), partly due to the differing preparation methods used by earlier studies. Cysts of *Pyrodinium* (a typical warm water species) were found to be

abundant and widespread along the southwest coast of Iran (Persian Gulf) in the study of Bradford and Wall (1984), but were totally absent along the southeast coast.

The cyst flora is dominated by *Scrippsiella* spp. followed by *Protoperidinium* spp. and *Gymnodinium* sp. Cysts of toxic species such as *Alexandrium* cf. *tamarense* and *L. polyedrum* were also found in the sediment especially in Pasabandar. This area can be considered as a potential 'seedbed' that could initiate blooms of toxic dinoflagellates.

6. Further Study

This study reveals cyst assemblages in the eastern part of Iran's south coast. The western south coast of the Gulf of Oman still requires investigation. Since the work of Bradford and Wall (1984) on the Persian Gulf (Iranian border), no other study on dinoflagellate cysts or other group on the Iranian coast has been carried out. Therefore investigation of dinoflagellate cysts along the entire south coast would help to provide a better understanding of the assemblage and composition of cyst flora, from a tropical area where environmental conditions of the Persian Gulf differ from the Gulf of Oman. In addition, further study could combine dinoflagellate cyst investigation with the assessment of phytoplankton populations. Ecological investigation of the cyst assemblage can be considered, with emphasis on effect of monsoon current and other physico-chemical parameters on cyst assemblage and abundance. In addition, the relationship of bloom toxicity and encystment, including assessment of factors inducing encystment or excystment of particularly toxic species, could be investigated.

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CHAPTER 3

Morphology and Phylogeny of a New Reticulate Cyst-Forming Dinoflagellate, *Gymnodinium trapeziforme* sp. nov.

1. Introduction

Three unarmoured Gymnodinioids have been reported to produce microreticulate cysts: *Gymnodinium catenatum* Graham (Anderson *et al.* 1988), *Gymnodinium nolleri* Ellegaard & Moestrup (Ellegaard & Moestrup 1999) and *Gymnodinium microreticulatum* Bolch & Hallegraeff (Bolch *et al.* 1999).

The first report of microreticulate cysts was from Spain in 1986 (Bravo 1986). However, it was not until Anderson *et al.* (1988) that these microreticulate cysts were identified as belonging to *G. catenatum* by the incubation of planktonic cysts collected during a bloom of this species from Galicia, in the north west of the Iberian Peninsula. Since then, microreticulate cysts have been reported around the world (e.g. Blanco 1995; Nehring 1995; Ellegaard & Moestrup 1999; Bolch & Hallegraeff 1990; Persson *et al.* 2000, Amorim *et al.* 2001; Wang *et al.* 2004). Similar cysts have also been recorded from areas where plankton cells have never been found in the water column (Bolch & Reynolds 2002), such as the Dutch North Sea coast (Nehring 1997).

Living cysts of *G. nolleri* were first described from Denmark in 1999 (Ellegaard & Moestrup 1999) and were subsequently found off the coasts of Sweden (Persson *et*

al. 2000), Italy (Montresor *et al.* 1998), Africa (Targarona *et al.* 1999), Somalia, Yemen and Pakistan (Zonneveld 1997).

Microreticulate cysts of *Gymnodinium microreticulatum* were first described from Australia by Bolch *et al.* (1999) and are known from the coasts of China (Qi *et al.* 1996), Portugal (Amorim *et al.* 2001) and Uruguay (Bolch & Reynolds 2002).

All three microreticulate *Gymnodinium* species produce cysts that are spherical and reddish brown (*G. catenatum* and *G. nolleri*), or pale to purplish-brown (*G. microreticulatum*) and show a distinctive microreticulate surface decoration (Anderson *et al.* 1988; Bolch *et al.* 1999; Ellegaard & Moestrup 1999). The arrangement of the network ornamentation reflects the amphiesmal vesicles of the vegetative cells comprising those defining the cingulum, sulcus and horseshoe-shaped apical groove (Bolch & Reynolds 2002). *Gymnodinium catenatum* is the only chain-forming and toxic dinoflagellate that causes Paralytic Shellfish Poisoning (PSP), while the other two species are not toxic.

Considerable efforts have been made over recent years toward a genetic description of *Gymnodinium* species, based on gene sequencing and the phylogenetic relationships of the species (see for example: Hansen *et al.* 2000a; de Salas *et al.* 2003). Ribosomal DNA can provide valuable phylogenetic affiliation for classifying the species (Adachi *et al.* 1996a, 1996b; Scholin *et al.* 1995). Each transcription unit in eukaryotes contains three genes: a small subunit (SSU, 18S), 5.8S and large-subunit (LSU, 28S) molecules which are separated by two internal transcribed spacers (ITS1 and ITS2) (Adachi *et al.* 1994).

During the present work examining resting cysts in modern sediments of southeast Iranian coast, some unusual, trapezoidal, angular, pale brown resting cysts were separated from sediments. The trapezoidal shape of the cyst is unique among living dinoflagellates cysts. Two live cysts were successfully germinated to produce a photosynthetic gymnodinioid dinoflagellate.

In this chapter, the morphology of the microreticulate cysts are described in detail, and the morphology and phylogeny of the vegetative cells of a new species,

Gymnodinium trapeziforme sp. nov., is described. Using large sub-unit ribosomal RNA gene sequences, this species is shown to be allied to, but morphologically and genetically distinct from, *Gymnodinium catenatum, Gymnodinium nolleri* and *Gymnodinium microreticulatum.*

2. Materials and Methods

2.1. Sediment Collection

Sediment samples were collected during March 2004 by the Iranian Fisheries Research Institute (IFRI)—Chabahar Centre, from 9 sites at three locations: the Bahoo-Kalat estuary, Pasabandar and Chabahar Bay, along the southeast coast of Iran (see Chapter 2, Fig.1 and Table 1 for site sampling details). Site selection was based on sediment characteristics and the previous occurrence of dinoflagellate blooms. Samples were collected by Ekman grab with a sampling area of 0.225m^3. The top layers of the samples were bottled and placed in the plastic containers, kept in the dark at 20°C, until transported to the laboratory for analysis.

2.2. Sediment Processing

Approximately 1-3g of sediment was taken from each sample and mixed with filtered seawater (FSW) in a clean 80ml plastic container to obtain a watery slurry. The sediment suspensions were sonicated for 2min using a Microson (200 watts) Ultrasonic cell disruptor to separate detritus particles. Samples were then filtered through a 125μm sieve and collected on a 20μm sieve using filtered seawater; the remaining parts were then panned to remove the denser sand grains and larger detritus particles (Bolch & Hallegraeff 1990). Sub-samples were then transferred into 14ml test tubes for the cyst concentration through a 2 step gradient density gradient centrifugation of Sodium Polytungstate (SPT) with specific gravities of 1.3 and 1.6× g cm^{-3} (Bolch 1997). Sub-samples of concentrated cysts were mounted and examined on glass slides for observation and identification of dinoflagellate cysts.

The relative abundance of each cyst type and quantitative estimates of cyst concentration were carried out as follows. A known wet weight of sediments were mixed with FSW then sonicated and sieved as described above. All sediments were carefully washed from the 20μm sieve into 45ml tubes and diluted to a suitable known volume. Living cysts were then concentrated through SPT at a specific gravity of 1.3×g cm^{-3} and the cysts resuspended in a known volume. The sub-samples (1ml) of processed samples were examined in a Sedgwick-Rafter chamber using an Olympus BH-2 microscope. The number of dinoflagellate cysts in each 1ml sample was counted and cyst concentration calculated (as cyst $gram^{-1}$ of wet sediment). A minimum of 100 cysts were counted from each sample.

2.3. Cyst Germination Experiments

Individual cysts recovered from the SPT processed samples were separated by micropipette under a Leica stereo-microscope (MZ95), and washed twice in growth media (GSe medium, Blackburn *et al.* 1989, see Appendix 1). The GSe medium was prepared using autoclaved filtered sea water of 35ppt salinity. Single cysts were then placed in a 55-mm polystyrene petri dish containing 15ml of GSe medium, sealed with parafilm and incubated at 26°C ± 0.5°C, under cool white fluorescent light (70-90μmol photons $m^{-1}s^{-1}$) with a 12h light: 12h dark cycle. Cysts were checked regularly for germination. After germination, cells were allowed to divide to form dense cultures that were transferred to the flasks containing 50ml GSe. The flasks were kept in the same condition as above for further experiments. Duplicate flasks of each strain were also held at 17°C ± 0.5°C at the same light intensity as above.

2.3.1. Encystment and Crossing Experiments

One millilitre of two cultured strains of *Gymnodinium* sp. (GYPC102, GYPC104), established from single cysts, were inoculated into petri dishes containing 20ml of a nitrate/phosphate-deficient GSe medium (Blackburn *et al.* 1989). The petri dishes were sealed with Para-film and incubated under the same conditions as above. Dishes were checked every two days for cyst production and the cysts produced were examined using light and electron microscopy.

To examine the mating behaviour of *G. trapeziforme*, ten individual cells were isolated from culture strain GYPC102 and incubated in 20ml of GSe media in the above conditions. After four weeks, each clonal strain was self-crossed by pipetting 1 ml of culture into 10ml of GSe media, without nitrate and phosphate, in 35mm petri dishes. Each clonal strain was also crossed in all possible pairwise combinations. All crosses were checked regularly for cyst formation.

2.4. Microscopy

2.4.1. Light Microscopy

Live and empty cysts were photographed with an Olympus BH-2 light microscope equipped with a Leica DC300F digital imaging system, an Axioskop 2 Plus microscope (Zeiss, Gotting, Germany) using bright field or differential interference contrast illumination.

2.4.2. Scanning Electron Microscopy (SEM)

Ten ml of mid-logarithmic growth-phase cultures of two strains of *Gymnodinium trapeziforme* (strains: GYPC102, GYPC104) were collected in 14ml centrifuge tubes, with the aim of stressing the cells as little as possible. The samples were gently centrifuged (1000×g) for 5min. The cells were then fixed by the addition of an equal volume of the concentrated cells (2ml) to 4% osmium tetroxide (OsO_4) in FSW and fixed for 30 min. (de Salas *et al.* 2004). Fixed cells were rinsed once with 70% FSW, and twice with the deionised water. The cells were then concentrated by centrifugation as above, adhered to polylysine coated cover-slips (Marchant & Thomas 1983) dehydrated in a graded methanol series (10-100% in eight steps), and critical-point dried (Balzers CPD 020, Germany) from liquid CO_2. The dehydrated coverslips were mounted on aluminium stubs, sputter coated with gold (Balzers SCP004, Germany) and examined using a JEOL (JSM-840) or Philips Quanta 600 scanning electron microscope at 20 KEV accelerating voltage.

Two methods were used for SEM of *G. trapeziforme* cysts. In the first method, the individual cysts were isolated from processed sediments, washed twice in distilled water, placed on 1μm Nucleopore filters, and air dried. In the second method, cultured cysts were concentrated by centrifuge and collected on poly-lysine coated coverslips and dehydrated in a graded methanol series (10-100%) as described above. Coverslips were then dried from hexamethyldisilazane (HMDL) in two steps, each taking 15 min. (Nation 1983). Stubs were sputter coated with gold and examined under a Philips Quanta 600 scanning electron microscope.

2.5. Toxin Analysis

Two hundred ml of culture strain GYPC102 in mid-logarithmic phase were harvested by gentle filtration onto 47mm Whatman glass-fibre filters. The filters were placed in 14ml tubes containing 2-4ml of 0.1N acetic acid, and sonicated on ice several times for 30 seconds each, using a Microson ultrasonic cell disruptor (200 watts) to homogenise the vegetative cells. All extracted samples were frozen at -20°C and transported to laboratory for PST toxin analysis.

Toxin analysis was kindly conducted by Dr Andrew Negri (Australian Institute of Marine Science). PST including C-toxins, gonyautoxin (GTX) and saxitoxins (STX) were analysed by HPLC method of Negri and Jones (1995). Briefly, according to Bolch *et al.* (1999), description of this method is as follows: extracted cells for water soluble toxin were centrifuged for 5 min. (6000 ×g) to separate glass-fibre and cell fragments. Toxins were separated using a Waters 600 HPLC, with a post-column reactor (Pickering PCX 5100) using 5μm, 250 × 4.6mm Alltima ODS column (Alltech, IL) with a flow rate of 0.8ml min^{-1}. Post-column oxidation was performed according to the method of Oshima *et al.* (1993). Derived PST fluorescence were detected with a linear LC305 spectrofluorometric detector (excitation at 330nm and emission at 390nm). The retention times and fluorescent intensity of the PSTs were compared with PST standards to identify PSTs. Identity of PST compounds were confirmed by removing post-column oxidation and observing the disappearance of peaks, and sample spiking experiments.

2.6. DNA Extraction and Polymerase Chain Reaction (PCR)

Approximately 10 ml of exponential growth phase cultures of *Gymnodinium trapeziforme* (GYPC102, GYPC104) was pelleted by gentle centrifugation (4000 rpm for 5 min), and the supernatant removed. DNA was extracted by the phenol:chloroform:isoamyl alcohol, gentle-lysis method, as described by Bolch *et al.* (1998) and resuspended in 200ml of sterile Milli-Q water or TE buffer. DNA quality was verified by 1% agarose-gel electrophoresis, stained with ethidium bromide and observed under UV light, using a high performance Ultraviolet transilluminator equipped with an Olympus digital camera. Sub-samples of resuspended DNA were diluted to an approximate concentration of 10 ng μl^{-1} and used as a template in PCR amplification. PCR primers D1R (Scholin *et al.* 1994) and 1483R (Daugbjerg *et al.* 2000) were used to amplify the D1-D3 (≈1500bp) regions of the large subunit ribosomal RNA gene (Table 1).

PCR reactions were performed in 50µl volumes in 200µl PCR tubes. PCR master mix contained 1×Bioline PCR buffer (160mM$(NH_4)SO_4$, 670mM Tris-HCL, 0.1% Tween-20), 1.5mM $MgCl_2$, 200µM dNTPs, 50pmoles of each Primer, 1U BioTaq DNA polymerase (Bioline, U.K) and 10 ng of template DNA. Thermocycling was as follows: initial denaturation of 2 min. at 94°C, followed by 35 cycles of 94°C for 1 min., annealing at 60°C for 1 min., elongation at 72°C for 2 min., and a final extension of 6 min. at 72°C. The PCR products were kept at 4°C prior to being stored at -20°C.

Five microliters of each completed PCR reaction was electrophoresed through 1% agarose gels to check for the presence of specific amplification products. Successful PCR products were prepared for sequencing by purification through Montage PCR cleanup columns (Millipore, USA) according to the manufacturer's protocols and then resuspended in sterile Milli-Q water.

Purified PCR reactions were then quantified by two methods: the first to estimate yield and purity; 2µl of each purified PCR product was subjected to 1% gel-electrophoresis and examined usually and compared to standard of known concentration. To determine more accurately the amount of DNA, a TBS-380 DNA

Fluorometer (Turner Biosystems, USA), was used following the manufacturer's protocol.

Table 1: Primer sequences used to amplify partial LSU of rRNA gene

Primer name/	Primer sequence (5'-3')
D1R-F[a]	ACC CGC TGA ATT TAA GCA TA
1483-R[b]	GCT ACT ACC ACC AAG ATC TGC

[(a)]Scholin *et al.* (1994)
[(b)]Daugbjerg *et al.* (2000)

2.6.1. DNA Sequencing

Purified PCR products were used as the template for sequencing reactions. The PCR products were sequenced using a Dye Terminator Sequencing Kit (Beckman-Coulter, Fullerton, CA, USA) following the manufacturer's protocols but using 40-60 ng of PCR product, 3.2pmol of primer per 10μl reaction, and the recommended cycle-sequencing parameters program. PCR products were sequenced in both directions using either the forward or reverse amplification primers in separate sequence reactions.

2.6.2. Alignment and Phylogenetic Analyses

Electropherograms obtained were checked by manual inspection using Bioedit (Hall 1999) and base-calling errors corrected manually. Sequence alignments were carried out with ClustalX version 1.83 (Jeanmougin *et al.* 1998), and improved manually. The PCR gel electrophoresis data and nucleotide alignment are shown in Appendices 2 and 3. Table 2 shows the taxa and their accession GenBank number where relevant that are used in the alignment. Unpublished sequences of Drs C. J. Bolch and M. F. de Salas are included in analyses for comparative purposes. These sequences are the subject of current research by Bolch and de Salas and will be submitted to Genbank on completion of other manuscripts not included here.

Table 2: List of species included in the phylogenetic analysis of partial LSU rRNA and GenBank accession

Species	Strain No.	GenBank Accession No.
Gymnodinium sp.	GY5HK	Bolch, unpublished
Gymnodinium sp.	GY5TRA	Bolch, unpublished
Cochlodinium cf. *geminatum*	CspTRA	de Salas, unpublished
Gymnodinium chlorophorum	K-0539	AF200669
Gymnodinium impudicum	JL30	AF200674
Gymnodinium cf. *impudicum*	GISR01	de Salas, unpublished
Gymnodinium nolleri	GNKB03	AY036079
Gymnodinium catenatum		
Gymnodinium microreticulatum	GMNC01	AY036078
Gymnodinium trapeziforme	GYPC102	Present study
Gymnodinium cf. *placidum*	K-0308	AF260383
Gymnodinium palustre	AJCKL-732	AF260382
Gymnodinium fuscum	AJC14-732	AF200676
Gymnodinium aureolum	S1-30-6	AF200670
Gymnodinium aureolum	Adel	de Salas, unpublished
Gymnodinium uncatenum	CS289	AY916541
Gymnodinium cf. *pulchellum*	GPKAWNZ	U92254
Gymnodinium uncatenum	GUDE00	Bolch, unpublished
Lepidodinium cf. *viride*		de Salas, unpublished
Polykrikos beauchampii		de Salas, unpublished
Polykrikos schwarzii	PSHK00	Bolch, unpublished
Polykrikos schwarzii	PSSH00	Bolch, unpublished
Polykrikos kofoidii	PKHK00	Bolch, unpublished
Akashiwo sanguinea	NEPCC355	AF260397
Gyrodinium dominans		AY571370
Gyrodinium rubrum		AY571369
Gyrodinium spirale		AY571371
Cochlodinium sp.	CPUNU01	de Salas unpublished
Gyrodinium falcatum	GFPLO1	AY320049
Gymnodinium sp.	GAPTB	de Salas unpublished
Karenia mikimotoi	Japan	de Salas unpublished
Karenia mikimotoi	CCMP429	de Salas unpublished
Karenia brevis		de Salas unpublished
Karenia papilionacea	G01HAWNZ	U92252
Karenia sp.	KPGB11	de Salas unpublished
Karenia sp.	KAPTB02	de Salas unpublished
Karenia bidigitata	CAWD80	AY947662
Karenia selliformis	G01FVXNZ	U92250
Karenia sp.	Chile	
Karenia brevisulcata		AY243032
Karenia umbella	KUTN05	de Salas unpublished
Karenia umbella	KULV01	de Salas unpublished
Karenia sp.		de Salas unpublished
Karlodinium micrum	G01WHKNZ	U92257
Karlodinium micrum	KDMPT01	AY263964
Karlodinium micrum	DB	de Salas unpublished
Karlodinium sp.	KDGT03	de Salas unpublished

Table 2 ***continued...***

Karlodinium sp.	KDTL11	de Salas unpublished
Karlodinium sp.	TKSB	de Salas unpublished
Takayama helix	THNWB01	de Salas unpublished
Takayama helix	THPA01	de Salas unpublished
Takayama tasmanica DW	CAWD114	AY947668
Takayama tasmanica TTTL02	CAWD115	AY947669
Woloszynskia sp.	WKT01	de Salas unpublished
Woloszynskia pseudopalustris	AJ12C1-915	AF260402
Woloszynskia sp.	CS341	AY916553
Togula compacta	K-0659	AY568562
Togula jolla	LB 1592	AY455680
Togula britannica	K-0658	AY455679
Alexandrium catenella	A3	AF200667

PAUP* version 4.0b10 for Macintosh (PPC) (Swofford 2002) for all phylogenetic analyses was used. For the analysis of LSU (D1-D3) rRNA sequences, the dataset contained 59 gymnodinioid taxa. The alignment comprised 1114 characters (including gaps introduced for alignment). *Alexandrium catenella* was used as an outgroup for the purpose of rooting the analyses. Significant phylogenetic structure was assessed by the random tree method using probability tables of critical value of g_1 (Hillis & Huelsenbeck 1992). Neighbour-joining (NJ) and maximum parsimony (MP) analyses were carried out to construct phylogenies from sequence alignment. Neighbour-joining trees were reconstructed with the minimum evolutionary (ME) method using LogDet distance (ME-LgD) as described by Bolch and Campbell (2004). For parsimony analyses, a heuristic search algorithm was used to find the most parsimonious trees, as analysis with 60 taxa used unreasonable amount of analytical time (months). Starting trees were generated by random addition of sequences. All characters were equally weighted and gaps were treated as missing data. To estimate the reliability of branch points, the MP and ME-LgD trees, bootstrap analyses (Felsenstein 1985) were carried out using 100 replicates of the full heuristic search algorithm.

3. Results

3.1. Description and Observations

***Gymnodinium trapeziforme* Attaran-Fariman & Bolch sp. nov.** **Figs 1-37**

Diagnosis:

Cellulae biconicae vel ovoideae, leviter dorsiventraliter compressae, 28-36 µm longae, 17-27 µm latae, 15-21 µm profundae. Cingulum aequatoriale descendens, profunde incisum, per circiter quintam partem longitudinis cellulae dislocatum. Sulcus in hypocono latus, in area intercingulari angustatus, et per circiter duas tertias partes epiconi ventralis extensus pro canale recto angusto. Canalis apicalis hippocrepiformis, a vicino sulci anteriori apicem circumiens contra horologii motum. Nucleus magnus, ovoideus vel lacrimiformis, in latere dextro cellulis, e medio hypoconi fere ad apicem extensus. Chloroplasti viridi-brunnei peripherales, irregulares vel taeniiformes. Cystae quiescentes trapeziformes vel subrectangulares, dorsiventraliter compressae, 26-24µm longae, 20-27µm latae, 8-10µm profundae. Cytoplasma globulare incolor, corpore accumulationis flavo-aurantiaco. Paries cystae pallide vel purpureo-brunneus, tectus polygonis elevatis in loco cinguli, sulci, et vesiculorum amphiesmalium cellulae vegetativae. Paracinguli latitudo circiter quartem partem cystae longitudinis. Archaeopyle chasmica, varie disposita.

Cells biconical to ovoid, slightly dorso-ventrally compressed, 28-36µm long, 17-27µm wide, and 15-21µm deep. Cingulum equatorially placed and descending, deeply excavated, displaced approximately 1/5 of cell length. Sulcus wide in the hypocone, narrowing in the intercingular region, and extending approximately 2/3 of the ventral epicone as a straight narrow groove. Apical groove horseshoe-shaped, extending from near the anterior sulcus and around the apex in anticlockwise direction. Nucleus large, ovoid to tear-shaped, positioned in the right side of the cell, extending from the middle of the hypocone to near the apex. Chloroplasts green-brown and peripherally placed, irregular to ribbon-like in shape. Resting cysts trapezoidal to sub-rectangular in outline and dorsoventrally compressed; 26-34µm long, 20-27µm wide, 8-10µm deep. Cytoplasm globular and colourless with an orange-yellow accumulation body; Cyst wall pale-brown to purple-brown, covered with raised polygonal reticulations reflecting the cingulum, sulcus and amphiesmal vesicles of the vegetative cell. Paracingulum width approximately 1/4 of the cyst length. Archeopyle chasmic, with variable orientation.

HOLOTYPE: Fig. 1; strain GYPC102
An individual cyst was isolated, germinated and cultured from coastal sediments collected near Pasabandar on the southeast coast of Iran in March 2004. Cultures are kept in the University of Tasmania, School of Aquaculture, Algae Culture Unit, Launceston, Australia.

TYPE LOCALITY: Pasabandar coast, Iran.

ETYMOLOGY: Named after the distinctive trapezoidal shape of the resting cysts of the species.

DISTRIBUTION: Southern coast of Iran, extending from near Pasabander to Bahoo-Kalat Estuary.

3.1.1. Vegetative Cell Description (Figs 1-15)

Gymnodinium trapeziforme is an unarmoured dinoflagellate with pale golden-brown coloured chloroplasts, found only as single cells in laboratory cultures. Cells are biconical to ovoid and dorsoventrally flattened (Figs 1, 2, 9 &10). In lateral view cells can have an ellipsoid outline (Figs 6 & 13). By light microscopy, the cell surface is smooth but under an electron microscope some specimens have a fine reticulate pattern reflecting the amphiesmal vesicles (Figs 9b, 11, 12 & 13). Cell size ranges from 27-38μm in length (average =33μm) and from 17-27μm in width (average=22μm). The epicone is conical with a round apex (Figs 1, 2 & 9a) and the hypocone is hemispherical (Figs 2 & 10), or sometimes biconical and truncated with an almost trapezoidal in outline (Figs 3, 7 & 9).

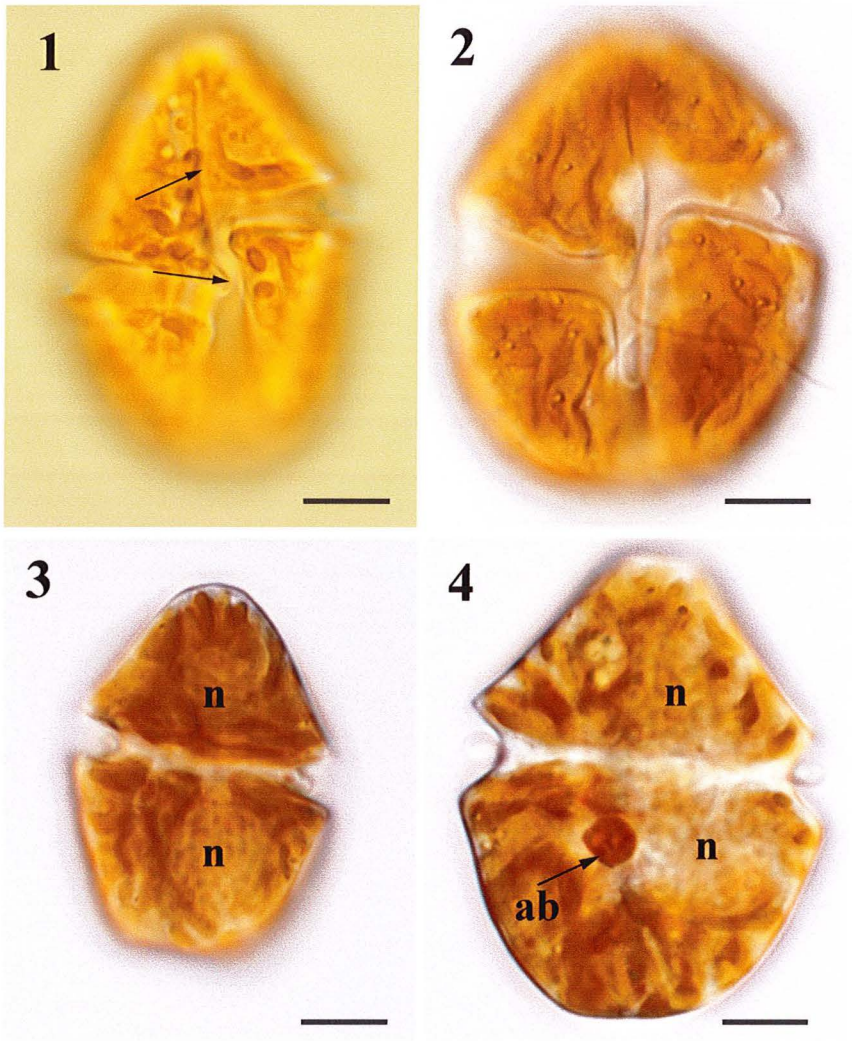
The deeply excavated girdle is median and forms a descending left spiral with a displacement of one-fifth of the cell length (Figs 1, 2, 9 & 10). In dorsal view, the cingulum is always straight and equatorially placed (Figs 7, 8 & 11).

The sulcus extends from approximately half way into the epicone and extends through to the antapex in the hypocone. It is narrow in the epicone and slightly wider in the hypocone, widening toward the antapex (Fig. 10). In the epicone, the right sulcal border is straight in the anterior part toward the apical groove, while, the posterior part is broader, narrowing near the anterior edge of the cingulum which protrudes into the sulcal area (Figs 1 & 2).

A horse-shoe shape apical (acrobase) groove begins from mid-epicone on the ventral surface close to the anterior end of the sulcus and encircles the apex in an anticlockwise direction terminating above and left of the anterior end of the sulcus (Figs 9a-b, 12, 13, 14 & 15).

A large pear-shaped to ovoid nucleus extends from mid-epicone to mid-hypocone (Figs 3, 4 & 5). A red-orange accumulation body was observed in some specimens in the hypocone near the dorsal left side of the nucleus (Fig. 4).

The chloroplasts appear green-brown under bright field illumination and are located in the periphery of the cell (Figs 5, 6, 7 & 8). The shape of the chloroplasts is generally ribbon-like and irregular (Fig. 7), to globular (Fig. 8). In lateral view, the chloroplasts appear to radiate from the apex and antapex toward the cingulum (Fig. 6), where the chloroplasts are oriented transversely, along the cingular margins (Figs 7 & 8).



Figs 1-4. LM. *Gymnodinium trapeziforme.*

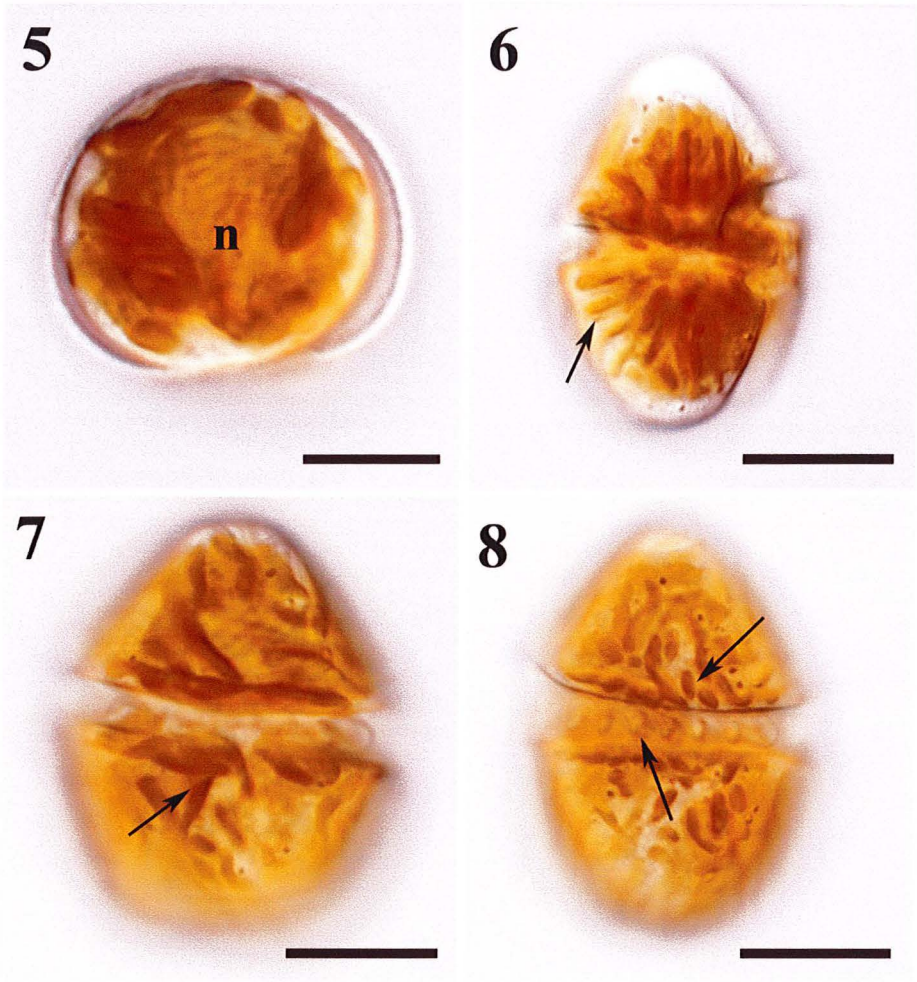
Fig. 1. Ventral view of cell, showing sulcal extension into the epitheca (top arrow) and small terminal protrusion or ventral flange (bottom arrow).

Fig. 2. Ventral view of the another cell (slightly compressed).

Fig. 3. Dorsal view of a small cell with the nucleus (n) situated in the right dorsal side of the cell, and extending above and below the cingulum.

Fig. 4. Dorsal view of a larger cell with a pear-shaped nucleus (n) occupying the right side of the cell. Accumulation body (ab) on the central left side.

All scale bars=10μm.



Figs 5-8. LM. *Gymnodinium trapeziforme.*

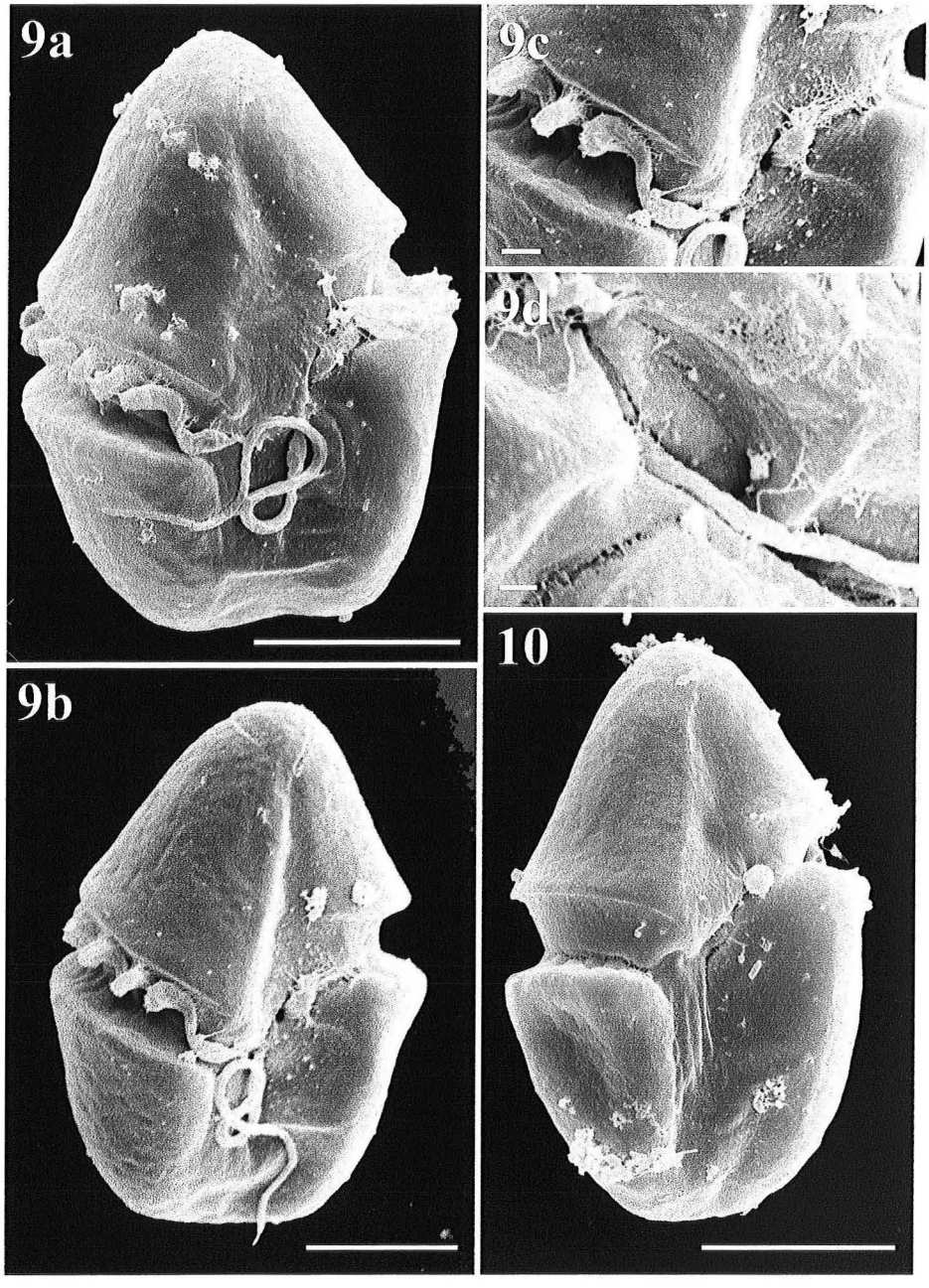
Fig. 5. Antapical optical cross-section of a cell showing the nucleus and peripheral chloroplasts.

Fig. 6. Lateral right view of cell, showing chloroplasts arrangements (arrow).

Fig. 7. Dorsal view of the cell with ribbon-shaped chloroplasts arrows.

Fig. 8. Dorsal view of the cell with globular chloroplasts (top arrows) and showing the transversely oriented chloroplasts along the cingular margins (bottom arrow).

All scale bars=10μm.

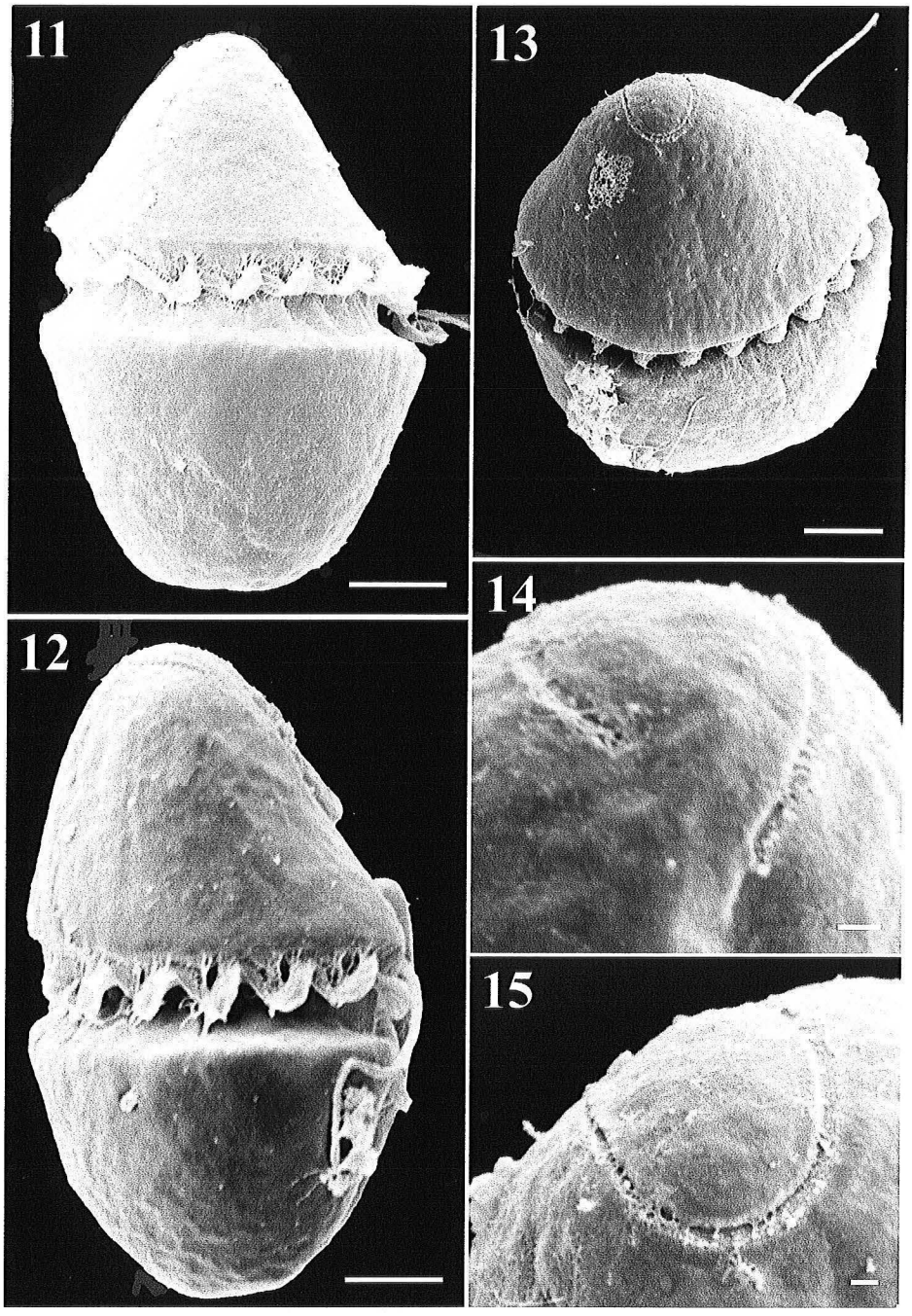


Figs 9-10. SEM. Motile cells of *G. trapeziforme.*

Fig. 9. Ventral view is showing conical epicone and trapezoidal hypocone with flattened antapex **(9a)**, reticulate surface **(9b),** transverse flagella within cingulum **(9c)**, and longitudinal flagella extending from sulcus **(9d)**.

Fig. 10. Typical cell shape in ventral view, showing the sulcal extension in epicone and hypocone. Note that some sulcal features of the live cells are not maintained in cells fixed for SEM.

All scale bars=10μm, except Figs 9c, 9d scale bar=1μm.



Figs 11-15. SEM. *Gymnodinium trapeziforme.*

Fig. 11. Dorsal view of cell.

Fig. 12. Lateral view, showing the apical groove.

Fig. 13. Apical view of the cell showing apical groove surrounding the apex.

Fig. 14. Ventral view of the cell showing horse-shoe shaped of acrobase that does not re-enter the sulcus in the opposite direction.

Fig. 15. Dorso-apical view of the cell showing the apical groove.

All scale bars=10μm, except Figs 13, 14 scale bars =1μm.

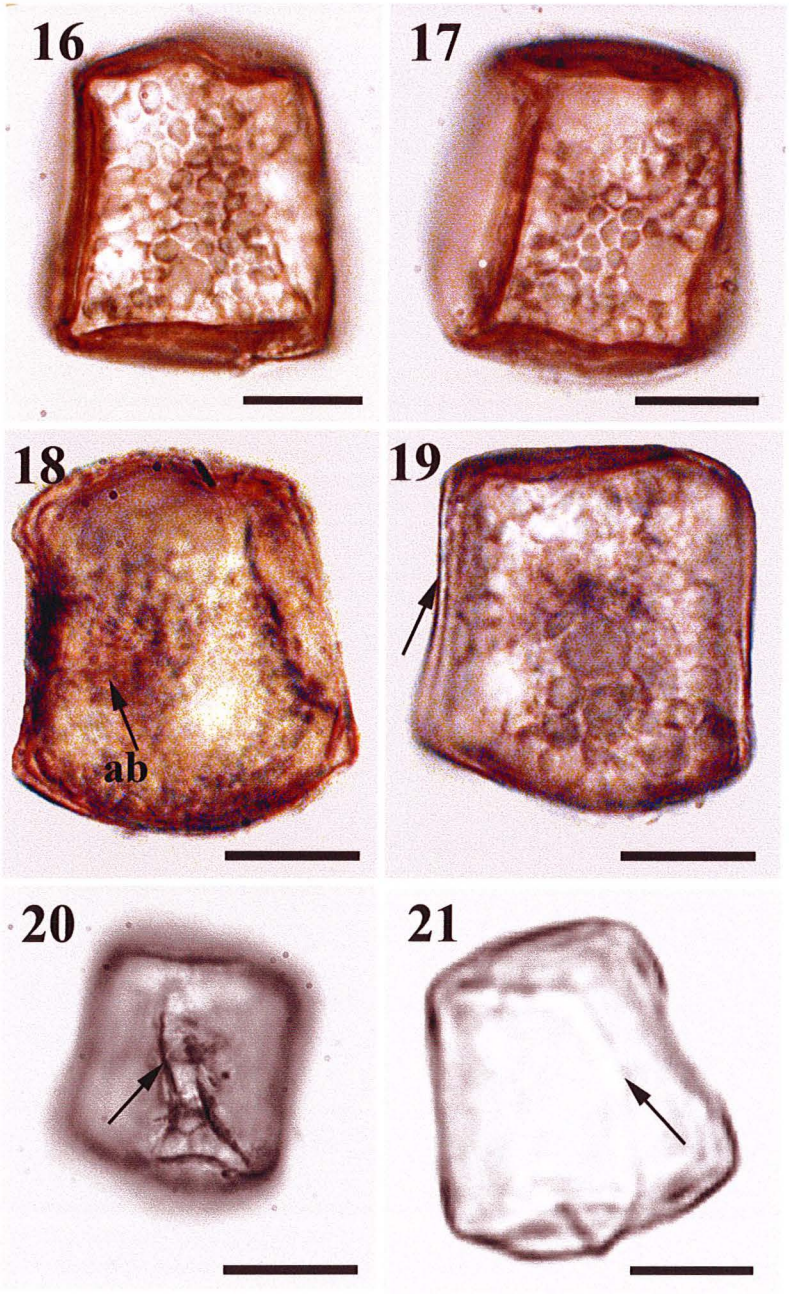
3.1.2. Resting Cyst Description (Figs 15-37)

The cysts of *Gymnodinium trapeziforme* are cubic-rectangular to cubic-trapezoidal, and laterally flattened. The size ranges from 17-27μm (average=21μm, n=30) in width, with a 2-7μm difference between the narrowest and widest point, and 23-34μm (average=26μm, n=30) in length. Live cysts have globular contents composed of clear lipid droplets. A dark yellow-orange accumulation body was observed in some specimens.

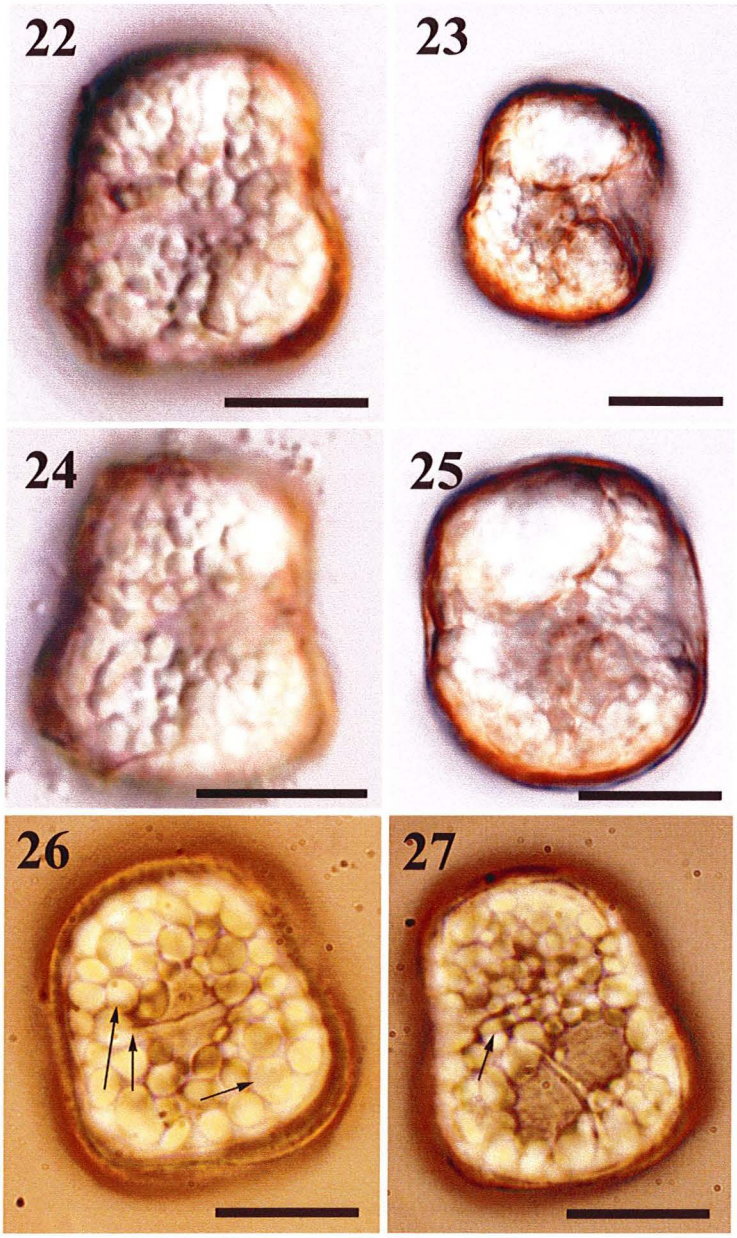
Intact wild cysts with cell contents are dark pinkish-brown to dark purple-grey in colour. Empty cyst walls are usually a pale-brown colour (Fig. 21). The cysts are almost rectangular in shape when examined by LM. (Figs 16, 17, 19, 20 & 21). They are straight or slightly concave on lateral borders, but may be round on the paraapices. The epicyst is slightly smaller than hypocyst (Figs 17, 18 & 21). Cysts sometimes appear cylindrical after fixation for electron microscopy (Fig. 28).

In comparison, cysts produced in laboratory culture are brown-green in colour and almost trapezoidal (Figs 24, 26 & 27). They are more or less straight in both paraapical and para-antapical outline, but are sometimes excavated in the median on the right or left sides of the cyst wall (Figs 23 & 24), with the corners of the cyst being more rounded (Figs 22 & 27). The epicyst is smaller than the hypocyst (Figs 24, 26 & 27). Interestingly, live cysts produced in cultures float at the surface of the growth medium.

When examined under SEM, both wild and cultured cysts walls are ornamented with polygonal microreticulations over their whole surface (Figs 28 & 30). The microreticulations are also visible on the wall, when viewed under a light microscope (Fig. 19).



Figs 16-21. LM. Resting cyst of *G. trapeziforme* from Iranian sediments.
Fig. 16. Live cyst showing almost rectangular shape and globular content.
Fig. 17. Lateral-epicystal view showing lateral compression.
Fig. 18. Wild cyst showing accumulation body (ab).
Fig. 19. Cyst showing reticulate outer wall (arrow).
Fig. 20. Surface focus in the live cyst showing a split running along the sulcus axis (arrow).
Fig. 21. Empty cyst with a sulcal-oriented archeopyle (arrow).
All scale bars=10μm.



Figs 22-27. Resting cyst of *G. trapeziforme* produced in laboratory cultures.
Fig. 22. Cyst showing almost trapezoidal shape with rounded corners.
Fig. 23. Cyst wall excavated around paracingulum.
Fig. 24. Trapezoidal cyst with smaller epicyst.
Fig. 25. Cyst showing a more rounded outline.
Figs 26-27. Cysts showing the protoplast (arrows).
All scale bars=10μm.

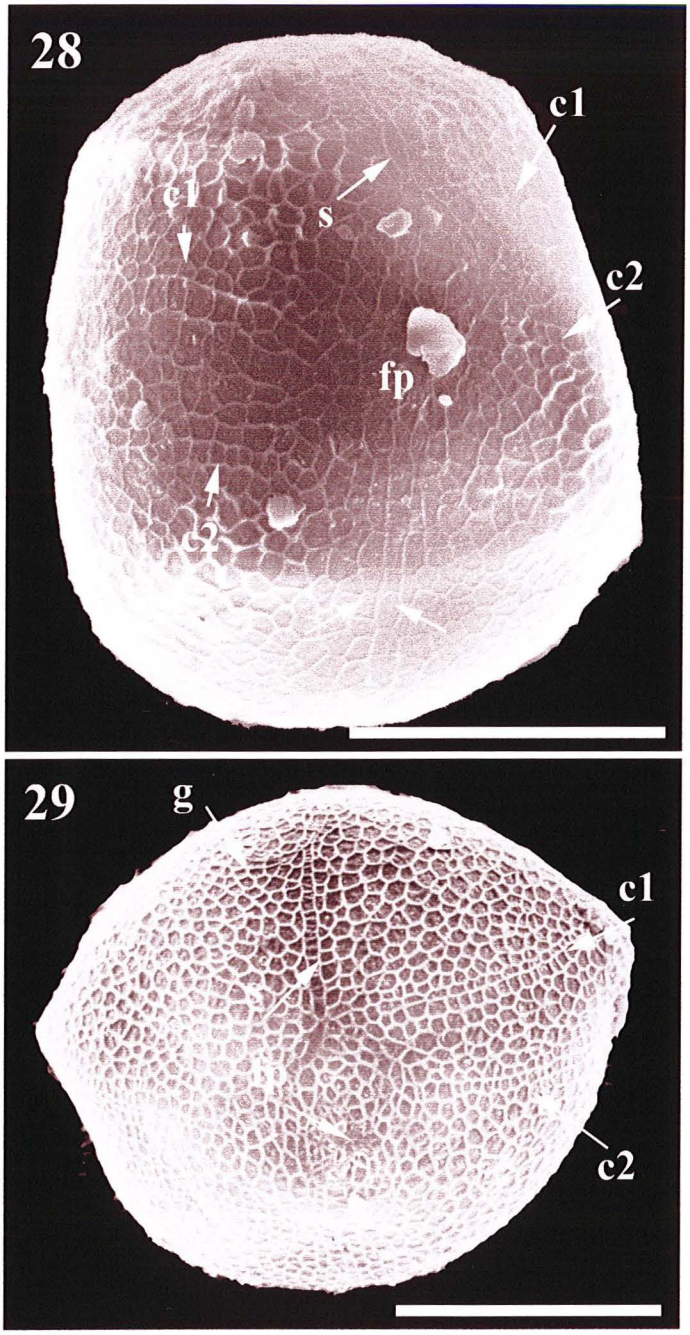
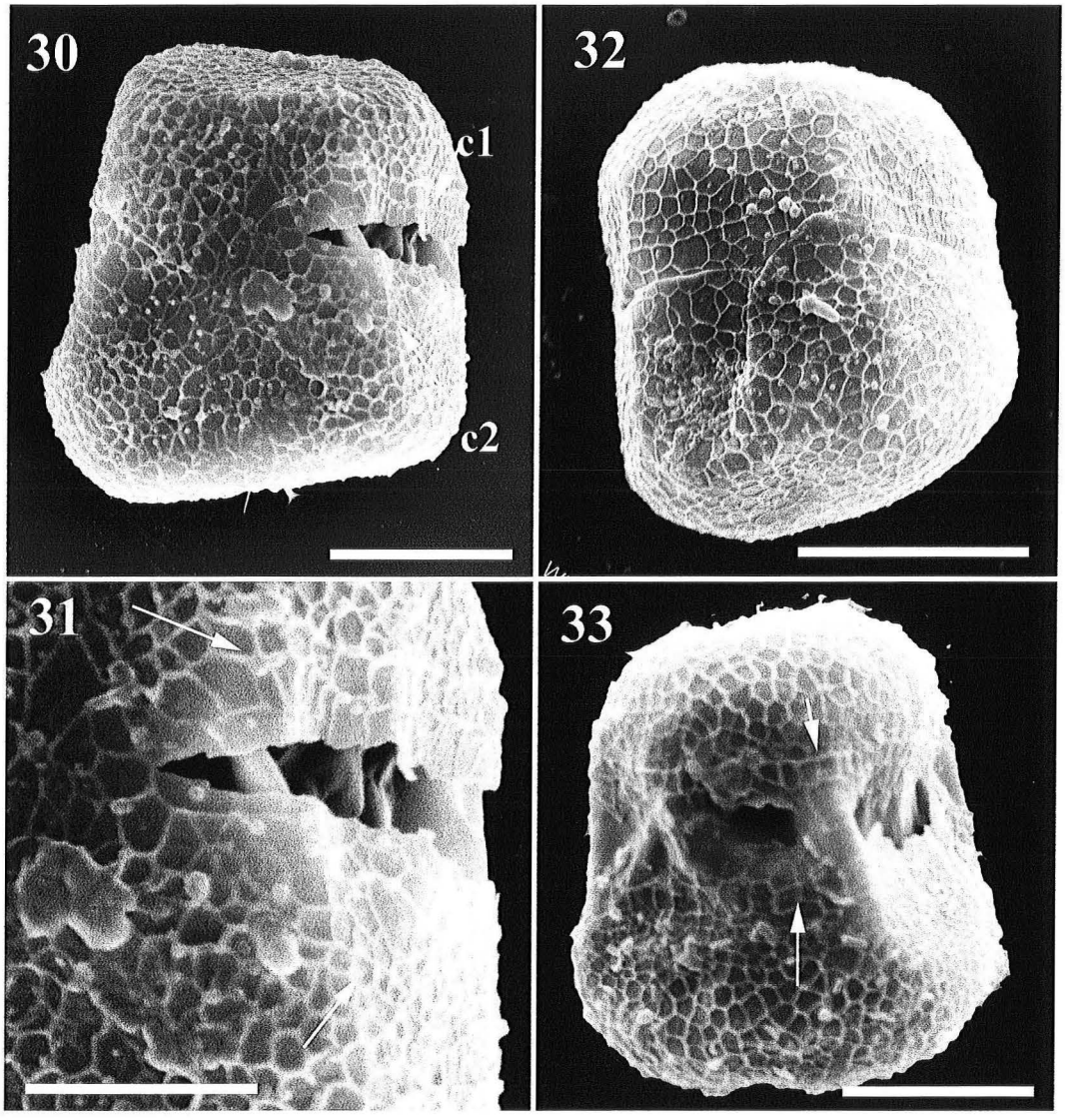


Fig. 28. SEM. Ventral view of *G. trapeziforme* wild cyst showing the paracingulum margins (c1, c2), parasulcus (s) and flagella pore region (fp).
Fig. 29. SEM. Typical microreticulate cyst (*Gymnodinium catenatum,* image from Rees and Hallegraeff, 1991) with a distictive paratabulation details.
Both scale bars=20µm.



Figs 30-33. SEM. Cultured cysts of *G. trapeziforme.*

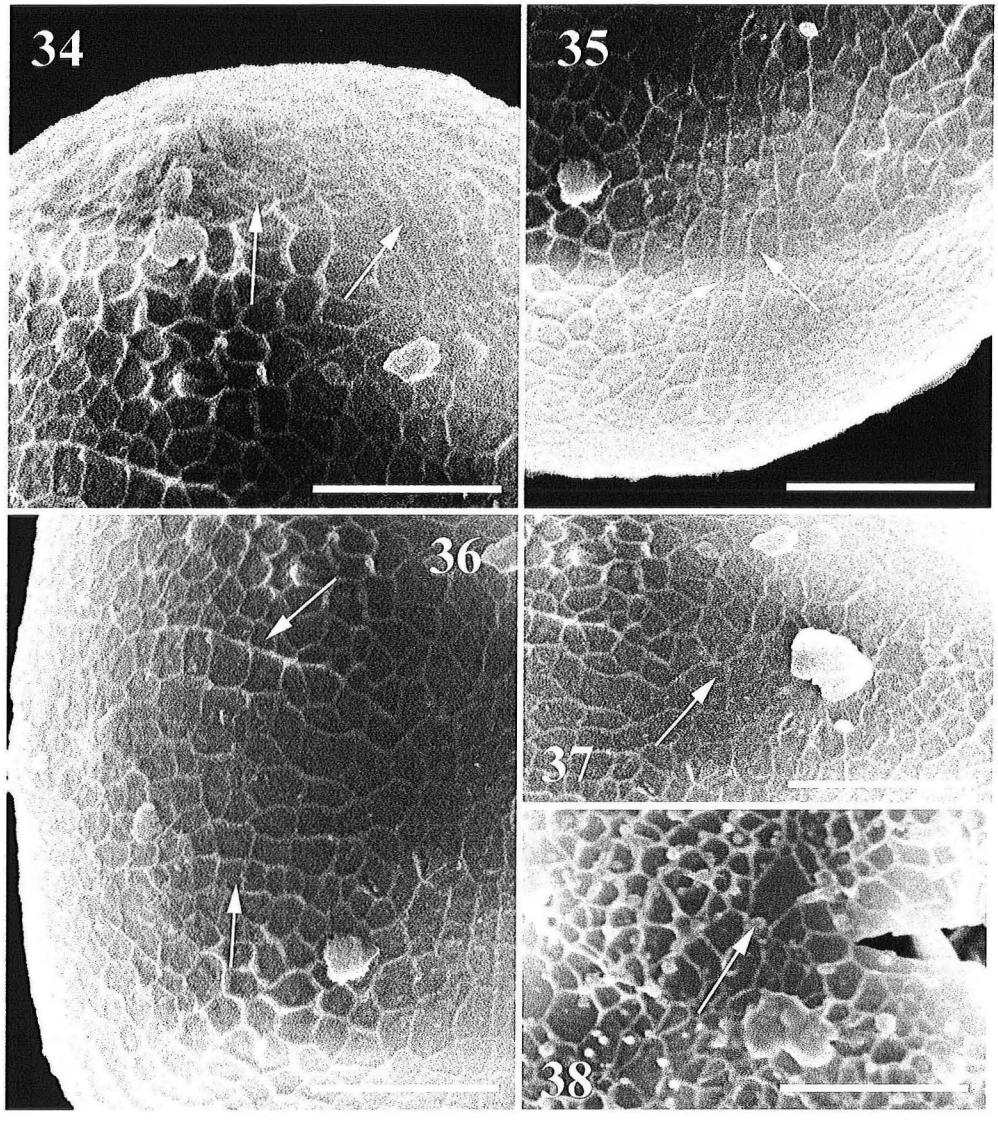
Fig. 30. Ventral view of cyst with clear paracingulum margins (c1, c2).

Fig. 31. Same cyst showing paravesicle pattern in the paracingulum borders (arrows).

Fig. 32. Dorsal view of cyst

Fig. 33. Dorsal view of cyst showing a cyst wall rupture along paracingulum (arrows). Note trapezoidal shape of the cyst.

All scale bars=10μm, except Fig. 31 scale bar=5μm.



Figs 34-38. SEM. *Gymnodinium trapeziforme* paratabulation details.

Fig. 34. Ventral view of epicyst with a flat row of paravesicles showing parasulcus (arrow) and possibly the para-acrobase (top arrow).

Fig. 35. Ventral view of hypocyst with two rows of flat paravesicles (arrows) defining the parasulcus.

Fig. 36. Ventral region of paracingulum. Note uniform margin of paravesicles along the paracingulum margin (arrows).

Figs 37-38. Ventral view of flagellar pore region of a wild cyst (Fig. 37) and cultured cyst (Fig. 38). Note larger size and irregular shape of paravesicles (arrows).

All scale bars=5μm.

The paracingulum is defined on both the apical and antapical borders by two rows of paravesicles with 3-4 rows of vesicles between cingular margins. On the epicystal side, the outer row is composed of smaller pentagonal or irregular flattened paravesicles (Figs 28, 30 & 36) that border an inner row of large, almost square paravesicles. On the hypocystal side, the paracingulum margin is formed by two rows of squarish paravesicles with the outer row being smaller paravesicles (Figs 28, 30 & 36). The parasulcus is delineated by two rows of flat rectangular paravesicles arranged in a straight line from near the antapex and a single row from the flagellar-root toward the apex. The paraapical groove is not clearly delineated. The paravesicles are larger in size and irregular in flagellar pore area (Figs 37 & 38). Germinated cysts possess an irregular chasmic archeopyle that is most often oriented along the parasulcal axis (Figs 30 & 33).

3.2. Nutrient Deficiency and Crossing Experiment

Cysts were produced in nutrient deficient culture after two weeks. The cysts showed the same reticulate pattern as wild cysts. However, some differences in the cyst shape and colour were observed as described above. Results of the crossing experiments showed that both self-crossed and crossed strains produced cysts (Table 3). The matrix of intercross and self-cross (Table 3) shows that five of ten strains produced resting cysts in self-crosses.

Table 3: Result of G. *trapeziforme* crossing-experiment

GYPC102	1	2	3	4	5	6	7	8	9	10
1	+									
2	+	-								
3	-	-	-							
4	-	+	-	+						
5	-	-	-	-	-					
6	+	-	-	-	-	+				
7	-	-	-	-	-	-	-			
8	-	-	-	-	-	-	-	-		
9	-	+	+	+	-	-	+	-	+	
10	-	+	+	+	-	-	+		+	+

- No cyst produced, + cyst produced

3.3. Toxins

HPCL analysis of PST toxins of *G. trapeziforme* (GYPC102) showed this species does not contain PST toxins. A standard chromatogram is shown in Fig. 39a. the few visible peaks in the Fig. 39b from strain GYPC102 are artefacts and not related to PST compound.

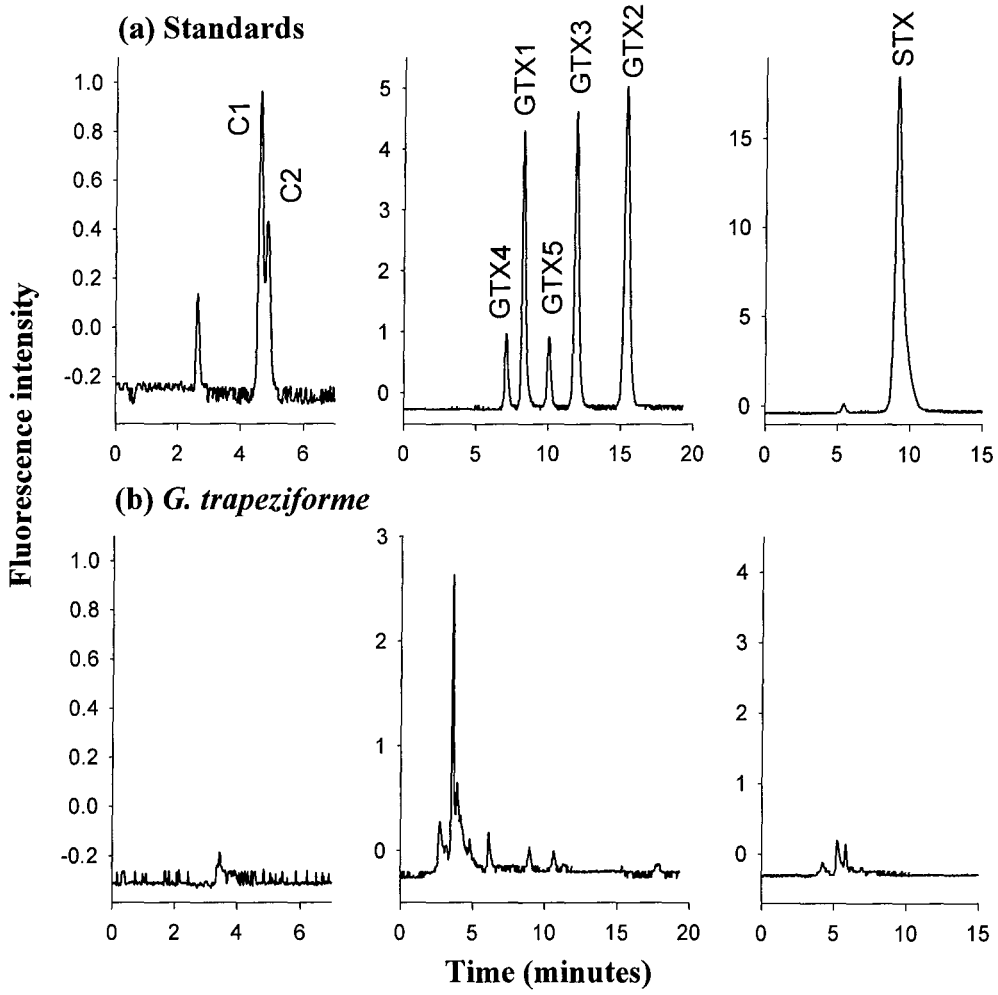


Fig. 39. High performance liquid chromatograms (HPLC) of post-column oxidation for the three classes of paralytic shelfish toxins: C-toxins, gonyautoxins (GTX) and saxitoxins (STX). (a) standard, (b) *G. trapeziforme*.

3.4. Phylogenetic Analyses of rRNA Genes

Evaluation of 10000 random trees from the dataset (Hillis & Huelsenbeck 1992) showed significant negative skewness of the distribution (ME-LgD model g_1= - 0.57, P<0.01). Therefore, the dataset contains phylogenetic signal and is more strongly structured than random data. The phylogenetic analyses of partial LSU-rRNA genes provide trees with almost same topology in both MP and ME-LgD distance analyses. The phylogenetic tree illustrated in Fig. 40 indicates 8 distinct clusters: *Gyrodinium*, *Gymnodinium*, *Karenia* Hansen &Moestrup, *Karlodinium* Larsen, *Polykrikos* Butschli, *Takayama* de Salas, Bolch, Botes & Hallegraeff, *Togula* Flø Jøgensen, Murray & Daugbjerg and *Woloszynskia*. The most parsimonious tree was found by heuristic search algorithms and is represented in Fig. 41. By removal of each of the clusters in turn, the branch topology remained the same.

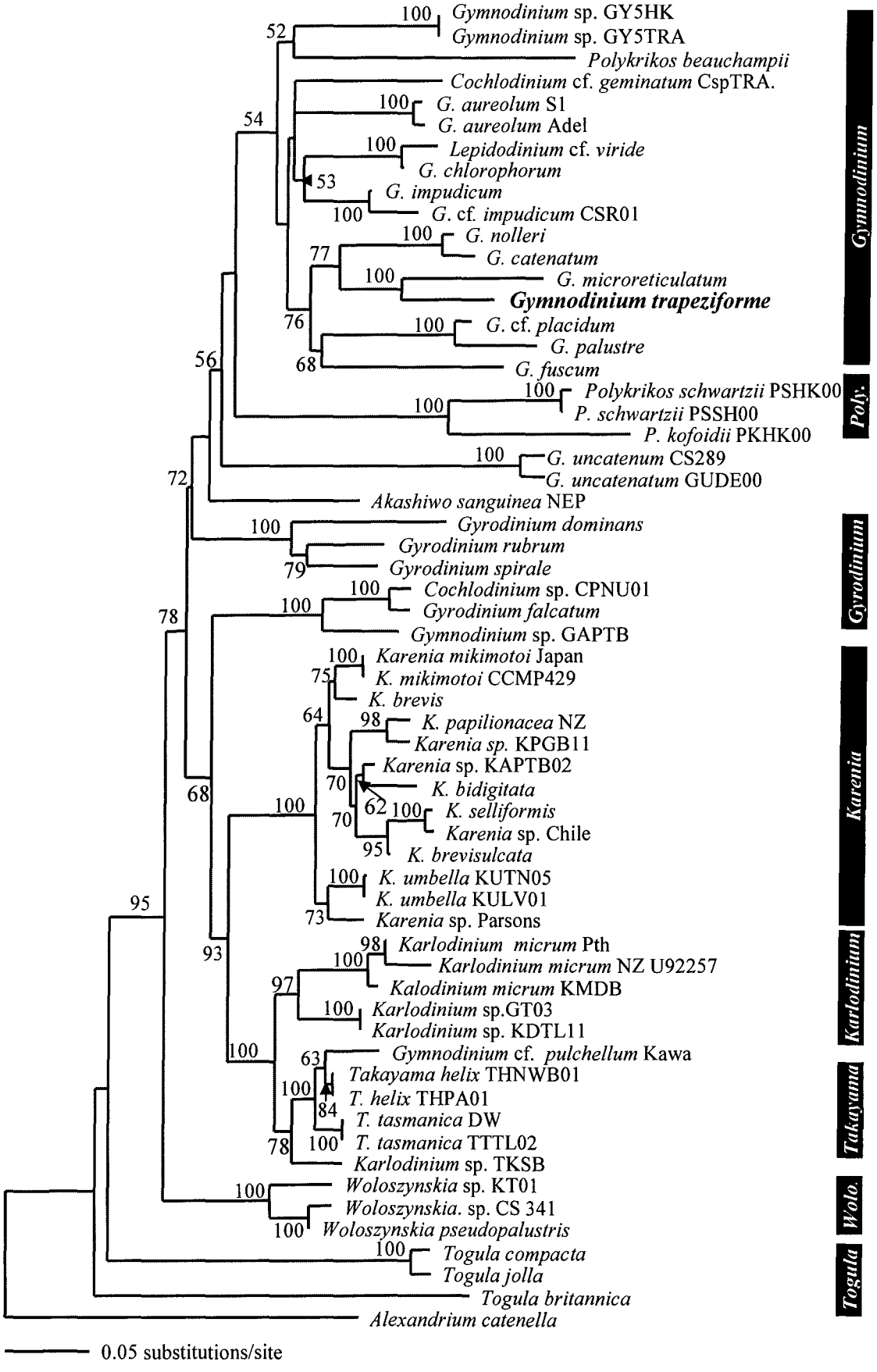
All molecular analyses show that the four microreticulate cyst-forming gymnodinioid are in the same monophyletic group. *G. trapeziforme* and *G. microreticulatum* fall within a monophyletic clade with the two other microreticulate cyst-forming species—*G. catenatum* and *G. nolleri*, with 77% bootstrap support in ME-LgD and 83% support in MP analyses (Figs 40 & 41). In this clade, *G. trapeziforme* was most closely related to *G. microreticulatum,* with 100% and 99% bootstrap support in ME-LgD and MP analyses respectively. Removal of each microreticulate cyst-forming *Gymnodinium* taxon did not affect the position of *Gymnodinium trapeziforme* in the analysis. The clade containing four *Gymnodinium* cyst-forming microreticulates cysts will be referred to as the reticulate *Gymnodinium* clade hereafter in this chapter.

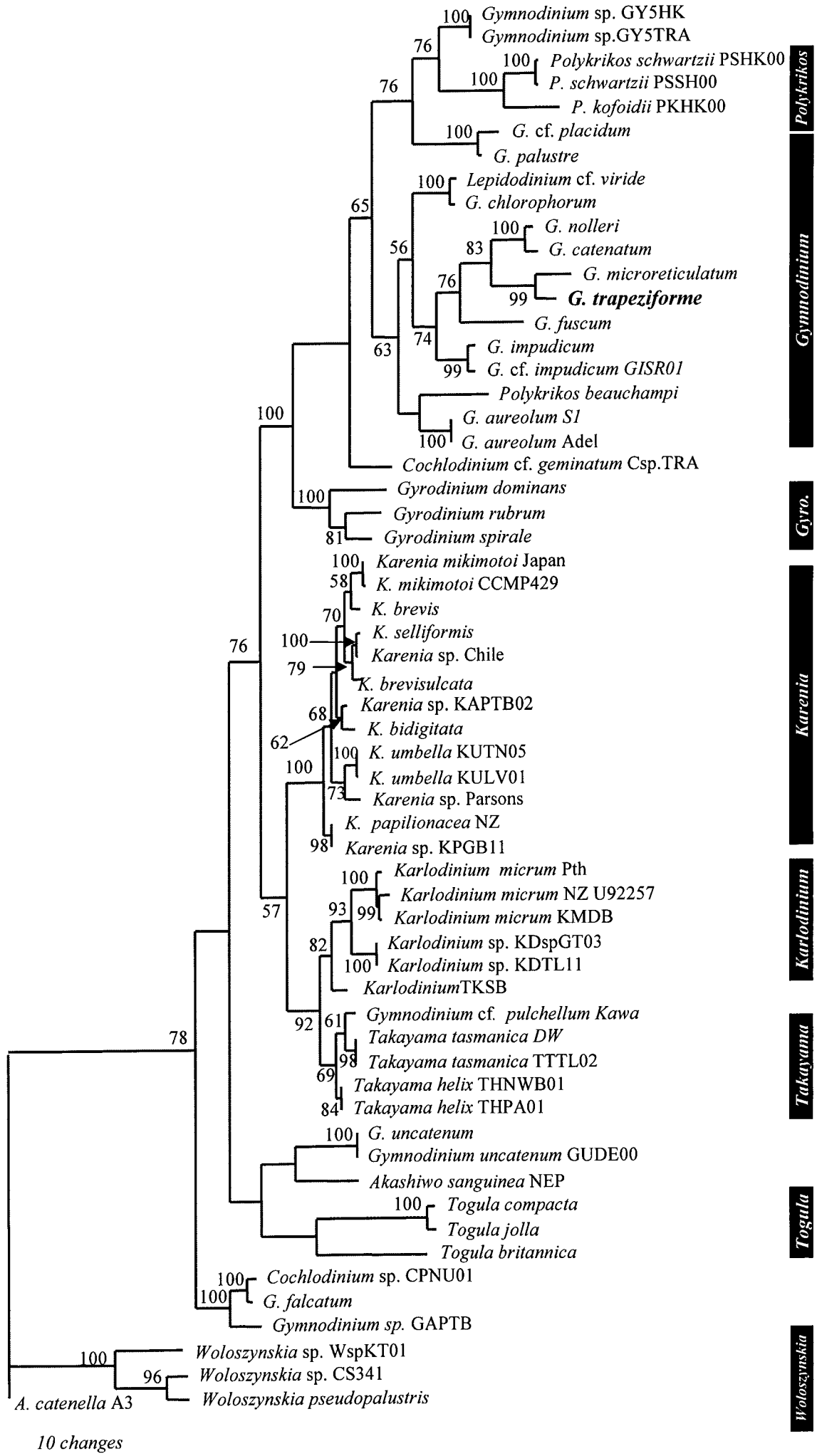
Table 4 presents a pairwise summary of genetic distances between *G. trapeziforme* and the other reticulate *Gymnodinium* species. Despite the fact that the sequence divergence of *G. trapeziforme* from *G. microreticulatum* and *G. nolleri* is 12.4% and 12.5% respectively (with less than 1% difference) (Table 4), the closest relative of *G. trapeziforme* is *G. microreticulatum* with 100% bootstrap support. The position of *G. trapeziforme* does not change even after removal of *G. microreticulatum*, it still groups with the remaining two species, but as the outside taxon and by removing *G. catenatum, G. nolleri* is still the outside taxon in the clade. The next most closely related group in the *Gymnodinium* cluster, consisting of three species: *G. fuscum*

(=type species), *G. palustre* and *G.* cf. *placidum* with 76% bootstrap support (Fig. 40).

Table 4: Pairwise sequence divergence (%) between four microreticulate cyst-forming species based on D1-D3 regions (930bp) of LSU rRNA

	G. trapeziforme	*G. microreticulatum*	*G. nolleri*	*G. catenatum*
G. *trapeziforme*	-			
G. microreticulatum	12.4	-		
G. nolleri	12.5	17.5	-	
G. catenatum	13.3	18.3	2.4	-


Gymnodinium sp. GY5HK
Gymnodinium sp. GY5TRA
Polykrikos beauchampii
Cochlodinium cf. geminatum CspTRA.
G. aureolum S1
G. aureolum Adel
Lepidodinium cf. viride
G. chlorophorum
G. impudicum
G. cf. impudicum CSR01
G. nolleri
G. catenatum
G. microreticulatum
Gymnodinium trapeziforme
G. cf. placidum
G. palustre
G. fuscum
Polykrikos schwartzii PSHK00
P. schwartzii PSSH00
P. kofoidii PKHK00
G. uncatenum CS289
G. uncatenatum GUDE00
Akashiwo sanguinea NEP
Gyrodinium dominans
Gyrodinium rubrum
Gyrodinium spirale
Cochlodinium sp. CPNU01
Gyrodinium falcatum
Gymnodinium sp. GAPTB
Karenia mikimotoi Japan
K. mikimotoi CCMP429
K. brevis
K. papilionacea NZ
Karenia sp. KPGB11
Karenia sp. KAPTB02
K. bidigitata
K. selliformis
Karenia sp. Chile
K. brevisulcata
K. umbella KUTN05
K. umbella KULV01
Karenia sp. Parsons
Karlodinium micrum Pth
Karlodinium micrum NZ U92257
Kalodinium micrum KMDB
Karlodinium sp.GT03
Karlodinium sp. KDTL11
Gymnodinium cf. pulchellum Kawa
Takayama helix THNWB01
T. helix THPA01
T. tasmanica DW
T. tasmanica TTTL02
Karlodinium sp. TKSB
Woloszynskia sp. KT01
Woloszynskia. sp. CS 341
Woloszynskia pseudopalustris
Togula compacta
Togula jolla
Togula britannica
Alexandrium catenella
Gymnodinium
Poly.
Gyrodinium
Karenia
Karlodinium
Takayama
Wolo.
Togula
0.05 substitutions/site


Gymnodinium sp. GY5HK
Gymnodinium sp.GY5TRA
Polykrikos schwartzii PSHK00
P. schwartzii PSSH00
P. kofoidii PKHK00
G. cf. placidum
G. palustre
Lepidodinium cf. viride
G. chlorophorum
G. nolleri
G. catenatum
G. microreticulatum
G. trapeziforme
G. fuscum
G. impudicum
G. cf. impudicum GISR01
Polykrikos beauchampi
G. aureolum S1
G. aureolum Adel
Cochlodinium cf. geminatum Csp.TRA
Gyrodinium dominans
Gyrodinium rubrum
Gyrodinium spirale
Karenia mikimotoi Japan
K. mikimotoi CCMP429
K. brevis
K. selliformis
Karenia sp. Chile
K. brevisulcata
Karenia sp. KAPTB02
K. bidigitata
K. umbella KUTN05
K. umbella KULV01
Karenia sp. Parsons
K. papilionacea NZ
Karenia sp. KPGB11
Karlodinium micrum Pth
Karlodinium micrum NZ U92257
Karlodinium micrum KMDB
Karlodinium sp. KDspGT03
Karlodinium sp. KDTL11
KarlodiniumTKSB
Gymnodinium cf. pulchellum Kawa
Takayama tasmanica DW
Takayama tasmanica TTTL02
Takayama helix THNWB01
Takayama helix THPA01
G. uncatenum
Gymnodinium uncatenum GUDE00
Akashiwo sanguinea NEP
Togula compacta
Togula jolla
Togula britannica
Cochlodinium sp. CPNU01
G. falcatum
Gymnodinium sp. GAPTB
Woloszynskia sp. WspKT01
Woloszynskia sp. CS341
Woloszynskia pseudopalustris
A. catenella A3
10 changes
Polykrikos
Gymnodinium
Gyro.
Karenia
Karlodinium
Takayama
Togula
Woloszynskia

4. Discussion

4.1. Morphological Comparison

The large genus *Gymnodinium* Stein was recently separated into four genera: *Gymnodinium* Stein emend. Hansen & Moestrup; *Gyrodinium* Kofoid & Swezy emend. Hansen & Moestrup; *Karenia* Hansen & Moestrup and *Karlodinium* Larsen (Daugbjerg *et al.* 2000). Species identification within the genus *Gymnodinium* is mainly based on the following features: shape and contours, size, chain formation, cingulum position and displacement, the presence and shape of the apical groove, sulcal extension into the epitheca, the sulcal-apical groove juncture, the shape of ventral ridge, the presence of striae, ribs, or furrows: the presence of chloroplasts and their colour and the size, shape and position of the nucleus (Steidinger & Tangen 1996).

Gymnodinium trapeziforme has a range of characters that position the species in the genus *Gymnodinium* such as: the species shape and contour and a horse-shoe shaped apical groove. Species in the genus *Gymnodinium* are defined as possessing a horseshoe-shape apical groove running anticlockwise around the apex: *G. fuscum* F Stein (= type species), *G. aureolum* (Hulburt) Hansen, *G. chlorophorum* Elbrachter & Schnepf, *G. impudicum* Fraga & Bravo, *G. palustre* Schilling, *G. maguelonnense* Biecheler, *G. catenatum*, *G. nolleri*, *G. microreticulatum* (Bolch *et al.* 1999; Hansen *et al.* 2000a; Daugbjerg *et al.* 2000). *G. palustre* is a freshwater species *G. fuscum* has a very weak apical groove and clearly different cell shape (Daugbjerg *et al.* 2000).

The cell shape, cingulum displacement, sulcal extension, loop-shape apical groove and chloroplast shape of *G. trapeziforme* most similar to *G. aureolum* (Hulburt) Hansen (Hansen *et al.* 2000a). *G. trapeziforme* usually shows a slightly sigmoid sulcus, and small protrusion of the epicone into the mid-sulcal region (Fig. 2) which is also comparable with that of *G. aureolum* (see Figs 22-24 & 26, Hansen *et al.* 2000a). However, this species differs from *G. trapeziforme* by having a colourless or

yellow-greenish and spherical to ovoid cyst type, and also in the shape and position of the nucleus. The nucleus of *G. aureolum* is wider than long and generally centrally positioned in the cell (Hansen *et al.* 2002a). *G. maguelonnense* Biecheler has a similar sulcal extension and cell shape to *G. trapeziforme* but this species also has a different nucleus shape and position and also radiating green chloroplasts. Little similarity can be found with *G. chlorophorum* either. It has a similar shape of apical groove and a descending cingulum (Honsell & Talarico 2004), however, it does not produce cysts and the chloroplasts are green rather than the typical brown-green dinoflagellate chloroplasts.

4.2. Comparisons with Microreticulate Cyst–producing Species

Reflected features of vegetative cells on the cyst wall (paratabulation) are quite common in armoured cyst-forming dinoflagellates (e.g. *Zygabikodinium lenticulatum)* and are a useful tool for species identification. In most unarmoured dinoflagellates, cyst morphology is not a useful character for species identification (Bolch *et al.* 1999). In the genus *Gymnodinium*, the type species *G. fuscum* produces a spherical cyst with a hexagonal or pentagonal pattern of ridges on the cyst wall (Hansen *et al.* 2000). Some species do not produce cysts (e.g. *Gymnodinium mikimotoi* Miyake & Kominami ex Oda) and some form a colourless, mucoid cyst without any distinctive morphological features (e.g. *G. aureolum*) (Bolch *et al.* 1999; Hansen *et al.* 2000a). However, the resting cysts of *G. catenatum*, *G. nolleri*, *G. microreticulatum* are distinct in their reticulate surface markings that reflect the acrobase, sulcus, and cingulum of the motile cell. Also the ability to fossilise makes these species distinctive among gymnodinioids (Bolch *et al.* 1999). Despite similarity in producing microreticulate cysts in all the above species, there are clear differences between the cyst and vegetative cells of these species. Details of morphological futures of the motile cell and cyst are summarised (Tables 5 and 6) and compared with other published microreticulate cyst-forming gymnodinioid species, *G. catenatum* (Anderson *et al.* 1988; Blackburn *et al.* 1989), *G. microreticulatum* (Bolch *et al.* 1999) and *G. nolleri* (Ellegaard & Moestrup 1999).

4.2.1. Vegetative Cell Comparisons

Single cells of the four species are similar in their general morphology (Table 5). All four species have similar horseshoe-shaped apical grooves and a deeply excavated left-handed cingulum. The sulcus of *G. trapeziforme* extends into the epicone, ending near the apical groove and does not re-enter the sulcus, similar to *G. microreticulatum, G. catenatum G. nolleri* (Bolch *et al.* 1999; Ellegaard & Moestrup 1999). Generally, the cell surface of all four species is smooth under the LM microscopy, but a honeycomb-like pattern of amphiesmal vesicles is seen under SEM. This pattern can also be seen in *G. trapeziforme* (Figs 11 & 13), but this reticulate network of amphiesmal vesicles was not as obvious as seen in similarly prepared cells of *G. catenatum* (Blackburn *et al.* 1989; Rees & Hallegraeff 1991). However, this may be due to the physiological state of the cells during fixation.

The vegetative cells of the four species: *G. catenatum*, *G. nolleri*, *G. microreticulatum* and *G. trapeziforme*, differ mainly in cell size, shape, chain-forming and the shape and position of the nucleus. Cell size of the *G. trapeziforme* is intermediate between *G. microreticulatum* and *G. nolleri,* but there is more overlap in size range with *G. microreticulatum* than *G. nolleri* (Fig. 42, drawing). The cell shape of the new species resembles *G. nolleri* with regard to its conical epicone and its rounded to cylindrical hypocone. *G. microreticulatum* has an ovoid to bi-conical shape (Bolch *et al.* 1999) and *G. catenatum* shows variety in shape: sub-spherical, bi-conical or squarish (Blackburn *et al.* 1989) depends on whether cells are in chains or not. All three species have a spherical nucleus (Table 5), however, the new species possess a large tear-shaped nucleus in the left lobe of the cell. Tangen (1977) and Hansen *et al.* (2000a) suggest that the position of the nucleus may vary in a species and they recommend that it be used as a taxonomic character only with caution, yet it is a useful character to distinguish these four species.

Table 5: Comparison of morphological features (motile cell) of the four microreticulate cyst-forming species

Motile cell features	*G. trapeziforme*	*G. microreticulatum*[a]	*G. nolleri*[b]	*G.catenatum*[c]
Size: Length	27-38μm	20-34μm	30-43μm	38-53μm
Width	17-27μm	15-22μm	23-33μm	33-45μm
Mean (L×w)	(33×22)μm	(17×24)μm	(33×26)μm	(45×26)μm
Girdle displacement	1/5 of body length	1/3-1/4 of body length	1/3-1/4 of body lengh	1/3-1/5 of body length
Apical groove	Horseshoe-like	Horseshoe-like	Horseshoe-like	Horseshoe-like
Nucleus	Pear-shaped, left side	Spherical in epicone	Spherical, central	Spherical, central
Chain-forming	Non-chain forming, single cell only	Non-chain forming, pairs of cells rare	Non-chain forming, paired Cells common	Chain-forming
Toxicity	Non-toxic	Non-toxic	Non-toxic	PSP toxins

(a), From Bolch *et al.* (1999)
(b) From Ellegaard & Moestrup (1999)
(c) From Anderson *et al.* (1988); Blackburn *et al.* (1989)

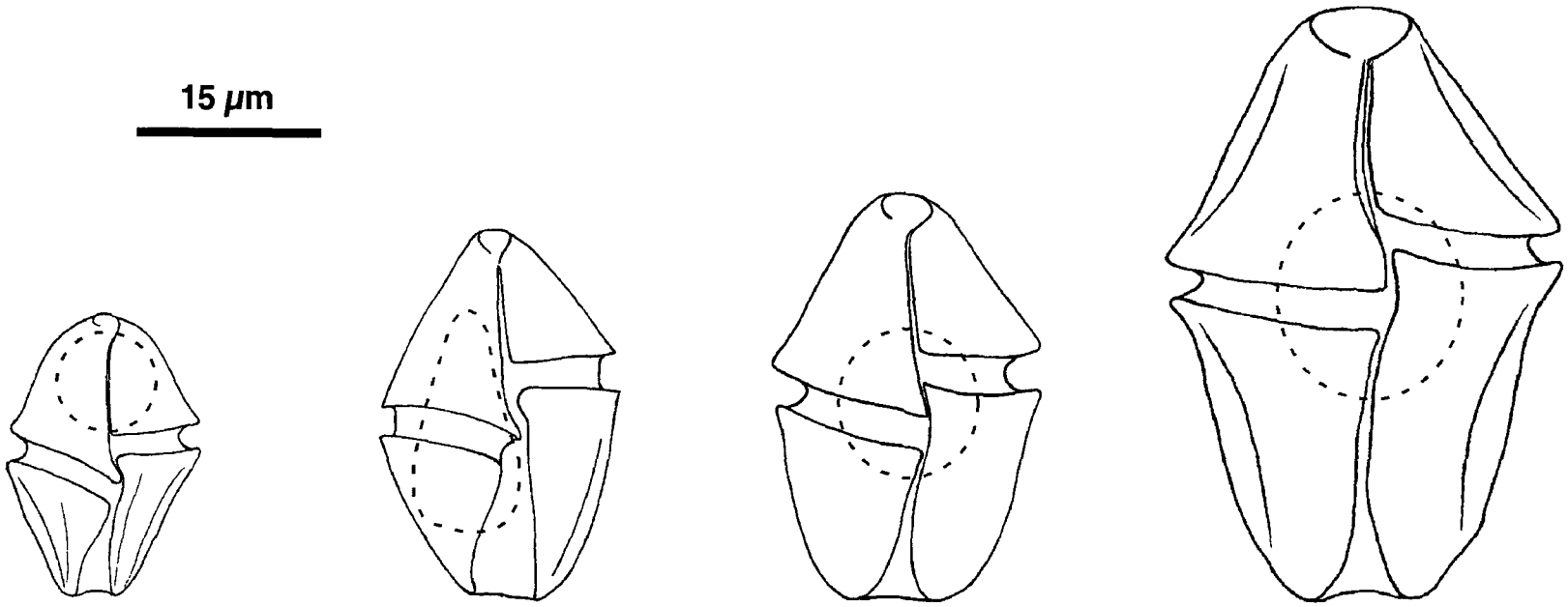


Fig. 42. Line drawing of single cell of four microreticulate cyst-producing gymnodinioids, comparing cell size, nuclear shape and position (modified from Bolch *et al.* 1999)
Scale bar=15μm

4.2.2. Cyst Comparisons

G. trapeziforme can be easily distinguished from the three previously known microreticulate cyst types by its unique shape. The cyst is sub-rectangular to trapezoidal compared to the spherical cysts of the other species. The purple-brown to brown-green colour of the *G. trapeziforme* cyst differs from both *G. catenatum* and *G. nolleri* that have a red-brown cyst wall colour. The colour is most similar the wall colour of *G. microreticulatum.*

In addition, there are differences in the reticulation pattern and size of the polygons—the size comparison is summarised in Table 6. *G. trapeziforme* more closely resembles *G. microreticulatum* and *G. nolleri.* However, in *G. trapeziforme*, the interparacingular paravesicles are larger in size, more irregular shape with possibly 3-4 rows. In contrast, *G. catenatum* possesses five to seven rows relative to bigger cyst size diameter (Amorim *et al.* 2001). However, there is doubt about the usefulness of reticulate pattern for separation of these microreticulate cyst types (Ellegaard & Moestrup 1999). The paravesiclar pattern defines the paraapical groove for all known previous cysts, whereas that of both wild and cultured cysts of the *G. trapeziforme* is not clear, and therefore, may need further investigation.

Cultured cysts of *G. trapeziforme* often tear in half straight along the cingulum (Figs 30 & 33), which is similar to cingular-oriented *G. catenatum* (Anderson *et al.* 1988) and *G. nolleri* (Ellegaard & Moestrup 1999). In natural sediments, live cysts and also empty cysts (Figs 20 & 21) demonstrated a split along the sulcal area, comparable with sulcal-oriented archeopyle of cultured cysts of *G. microreticulatum* (Bolch *et al.* 1999). It is not clear whether the split in the culture cyst of *G. trapeziforme* is a true archeopyle. However, Bolch *et al.* (1999) discussed that there is also some inconsistency in the orientation of archeopyle in the other reticulate cyst types.

Tabl 6: Comparison of morphological features of cysts in the four microreticulate cyst species

Cyst features	*G. trapeziforme*	*G. microreticulatum*[a]	*G. nolleri*[b]	*G. catenatum*[c]
Shape	Squarish to trapezoidal	Spherical	Spherical	Spherical
Size	Width:17-27μm, Length:23-34μm Depth: 8-10μm	Diameter:17-28μm	Diameter: 28-38μm	Diameter: 38-58μm
average	21×26μm	24μm	31μm	49μm
Colour	Purple-brown	Pale purple-brown	Red-brown	Red-brown
Accumulation body	Yellow-orange	Pale yellow-orange	Dark red	Dark red
Girdle displacement	1/4 of cyst length	1/4 of cyst diameter	1/4 of cyst diameter	1/5 cyst diameter
Polygon size	0.6-2.2μm	0.8-2μm	0.3-1.9μm	1-3μm
Archeopyle	Chasmic, often along parasulcus (wild cyst)	Chasmic, often along parasulcus	Chasmic, often along paracingulum	Chasmic, often along paracingulum

(a-), From Bolch *et al.* (1999)
(b)- From Ellegaard & Moestrup (1999)
(c)- From Anderson *et al.* (1988); Blackburn *et al.* (1989)

4.3 Phylogenetic Relationships

From the analyses presented here, the closest relative of the reticulate gymnodinioids is the clade that includes *G.* cf. *placidum, G. palustre* and *G. fuscum*. From morphological similarities, *G. aureolum* is most similar to these species (Hansen *et al.* 2000a) along with *Gymnodinium impudicum* and the green species *Gymnodinium chlorophorum.* Daugbjerg *et al.* (2000) also reported that the ultrastructure of *G. aureolum* is very similar to that of *G. catenatum* and *G. nolleri*. However, this is not supported by the phylogenetic analyses here (Fig. 40, log-Det tree). Due to differences between *G. fuscum* and its relatives from other members of the *Gymnodinium* genus, doubts have been raised by Daugbjerg *et al.* (2000) about the separation of these species from the large genera of *Gymnodinium*; however, they suggested further enquiry. The closest relatives of the microreticulate gymnodiniums remain unclear. Resolution of this issue may require the addition of a wider range of species to phylogenetic and morphological analyses.

4.4 Crossing and Cyst Formation

Preliminary crossing experiments reveal that *G. trapeziforme* may have a homothallic mating system. Many dinoflagellates have a simple homothallic mating system (e.g. *Scrippsiella trochoidea*, Montresor *et al.* 2003) or a simple binary (+)/(-) heterothallic mating system (e.g. *Alexandrium catenella*, Yoshimatsu 1984). Some species such as *Alexandrium tamarense* have a multiple mating group system including both heterothallic and homothallic strains (Destombe & Cembella 1990). In reticulate gymnodinioids, *G. catenatum* and *G. nolleri* are similar in both having a complex heterothallic mating system, although they show some clear differences (Blackburn *et al.* 2001; Figueroa & Bravo 2005). *G. catenatum* also shows homothallic mating. Some cultures established from a single cysts are able to self-cross and produce resting cysts (Blackburn *et al.* 2001). In contrast, *G. nolleri* never produced cysts from self-crosses (Figueroa & Bravo 2005). On the other hand, preliminary study on mating type of two other reticulate gymnodinioids: *G. microreticulatum* and *G. trapeziforme* suggest that both have simple homothallic mating systems (Bolch *et al.* 1999; present study).

5. Conclusion

The motile cell and resting cyst of *Gymnodinium trapeziforme* were described and this species referred to the genus *Gymnodinium*. Based on morphological and molecular affinities, the new species is part of the microreticulate cyst-producing group that includes *G. catenatum,* but is genetically and morphologically distinct from the other members of the group. The cyst of *Gymnodinium trapeziforme* is distinct from the three other microreticulate cyst-forming species: *G. catenatum*, *G. nolleri*, and *G. microreticulatum*. It is easily distinguished by the squarish to trapezoidal cyst shape, the motile cells that do not form chains, and possession of a tear-shaped nucleus.

6. Further Study

Ultrastructural studies of dinoflagellates (for example: Rees & Hallegraeff 1991; Hansen 2001; Hansen & Daugbjerg 2004; Hansen & Moestrup 2005) indicate that flagellar apparatus may be highly conserved and therefore should be a supportive criteria in taxonomy and phylogenetic relationships of naked dinoflagellates. Therefore, it would be helpful to investigate the flagella apparatus of the new species and to compare it with other reticulate cyst-forming *Gymnodinium* species, as well as other *Gymnodinium* species including *G. fuscum*. In addition, the following further research should be considered: investigation of the chloroplast pigments, compared with other members of the group; assessment of vegetative cells from the water column; ecological investigation of both cyst and motile forms in the natural sediment; lifecycle of the species and also investigation of the other coastal areas along the south coast of Iran and Persian Gulf. The effects of physio-chemical factors and monsoon currents on encystment and cyst distribution can also be investigated. Furthermore, as cysts showed a high relative abundance in locations associated with annual HABs, it should be investigated whether this is a bloom former species, as well as the cyst's role as a bloom initiator.

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CHAPTER 4

Morphological and Molecular Analysis of *Scrippsiella* species Isolated from the Southeast Coast of Iran

1. Introduction

The genus *Scrippsiella* Balech & Loeblich III is a morphologically conservative but ecologically diverse group of thecate photosynthetic marine dinoflagellates. Species classification in the genus is based on acombination of morphology of the vegetative cells (i.e. thecal plates) and cysts (if known) and more recently assisted by molecular data. In the classification system proposed by Fensome *et al.* (1993), this genus and *Ensiculifera* Balech belong to the subfamily Calciodinelloideae Fensome *et al.* (1993) (Order Peridiniaceae Ehrenberg). The subfamily includes about 30 extant species; a fossil genus, *Calciodinellum* Deflandre, is the type genus of the subfamily.

Species belonging to the Calciodinelloideae generally produce calcareous resting cysts, however, some species produce organic-walled cysts, such as *Scrippsiella hangoei* Schiller (Larsen *et al.* 1995) and *Ensiculifera imariense* Kobayashi & Matsuoka (Kobayashi & Matsuoka 1995). *Pentapharsodinium* Indelicato & Loeblich, which also produces calcareous cysts, was considered by Fensome *et al.* (1993) as an uncertainly placed because the cysts lacked calcareous ornamentation. Subsequently, Montresor *et al.* (1993) described the calcareous cyst of *Pentapharsodinium tyrrhenicum*, and it is now clear that species of all three extant genera produce can calcareous cysts (Montresor 1995; Matsuoka & Fukuyo1995; D'Onofrio *et al.* 1999). In addition, three additional genera were established to

describe fossil other calciodinellid cysts; *Calciodinellum* Deflandre, *Calcigonellum* Deflandre and *Pernambugia* Janofske & Karwath (Gottschling *et al.* 2005a, 2005b). More recently, however, living resting cysts and motile cells consistent genera have been discovered that correspond with these initially fossil genera (e.g. *Calciodinellum levantinum* Meier Janofske & Willems from the Mediterranean Sea; Meier *et al.* 2002).

Calcareous dinoflagellate cysts are common and widely distributed from temperate coastal areas (Ellegaard *et al.* 1994; Montresor 1995; Blanco 1995; Larsen *et al.* 1995) to sub-tropical oceanic environments (Wall & Dale 1968; Dale 1992; Zonneveld 1997; Wendler *et al.* 2002). Fossilised calcareous cysts are also well known from the Upper Triassic onwards (Janofske 1992), and are abundant during the Cretaceous (Willems 1994) and Eocene (Kohering 1993). Some fossils cyst types have also been recorded from recent surface sediments (e.g. Wall & Dale 1968; Montresor *et al.* 1994) and can provide a valuable link between living and fossil species.

Species belonging to the genus *Scrippsiella* are one of the most important components of dinoflagellate flora in coastal waters (Honsell & Cabrini 1991; Faust 1996). The genus currently includes about 20 extant species (Gottschling *et al.* 2005a); with nine species known to produce calcareous cysts (Montresor 1995). *Scrippsiella* species are relatively small autotrophic cells with resting cysts that often dominate the cyst flora in coastal areas (e.g. present study; Qi *et al.* 1996; Joyce 2004). The cysts are also found in sediment and sediment trap material worldwide (Bolch & Hallegraeff 1990; Dale 1992; Montresor *et al.* 1998; Kim & Han 2000; Janofske 2000; Godhe *et al.* 2001; Montresor *et al.* 2003). The diversity and abundance of *Scrippsiella* cysts is higher in tropical and sub-tropical regions than temperate coastal waters (Vink 2004), yet our knowledge of the diversity and abundance of *Scrippsiella* in the tropical phytoplankton is very limited.

Cyst wall composition and structure, archeopyle type, cyst shape, and the number of thecal plates on the vegetative cell are the main taxonomic features used in the classification of calcareous dinoflagellates cyst species (Fensome *et al.* 1993;

D'Onofrio *et al.* 1999). For resting cysts, the shape and arrangement of calcareous crystalline processes are important features for taxonomy (Lewis 1991).

Many calcareous resting cysts germinate to produce small motile cells with a typical *Scrippsiella* plate pattern and have generally been referred to *S. trochoidea* (Blanco 1995; Montresor *et al.* 1998; Godhe *et al.* 2000). However, Montresor *et al.* (2003) has demonstrated high morphological, physiological and genetic variability within this single morphospecies, suggesting the presence of several unresolved cryptic species. The separation of cryptic species within the group based only on cell morphology can be difficult or impossible, therefore, the combination of molecular data with cyst and germinated cell morphology with can be a valuable aid for distinguishing *Scrippsiella* species.

Ribosomal RNA gene sequences have been useful for examining the phylogenetic relationship among a great diversity of taxa, because of the presence of both highly conserved and variable regions (e.g. Adachi *et al.* 1994, 1996a, 1997; Scholin *et al.* 1994). Nucleotide sequences of internal transcribed spacers (ITS) regions containing the 5.8S rRNA gene are considered to be less conserved than the small and large subunit rRNA genes and are therefore useful for phylogenetic inference at the inter- and intraspecific level (Adachi *et al.* 1994; Adachi *et al.* 1996b; Adachi *et al.* 1997; Penna & Magnani 1999). This region has also been shown to be suitable for evaluation of genetic relationship among *Scrippsiella* species (Montresor *et al.* 2003; Gottschling *et al.* 2005a, 2005b). In this chapter I describe the cyst and cell morphology of four distinct *Scrippsiella* species from the south coast of Iran: *Scrippsiella irregularis* sp. nov., *Scrippsiella trochoidea*, *Scrippsiella* sp. 1 and *Scrippsiella* sp. 2. Sequences of the rDNA-ITS of the four species are also compared with previously described, closely related species where possible. This work is the first report of *Scrippsiella* species from the southeast coast of Iran.

2. Material and Methods

2.1. Sediment Collection and Processing

Nine sediment samples collected from three stations and processed as previously described in Chapter 3.

2.2. Cyst Germination Experiments

Individual cysts were isolated from processed, washed sediment suspensions using a micropipette under a Leica stereo-microscope and washed twice in sterile growth media of 35ppt salinity (GSe medium, Blackburn *et al.* 1989, see Appendix 1). Washed cysts were then placed in 55mm polystyrene petri dishes containing 15ml of GSe medium, sealed with parafilm and incubated at 26°C ± 0.5°C, under cool white fluorescent light (70-90μmol photon $m^{-1}s^{-1}$) with a 12h light: 12h dark cycle. The remaining mixed sediments were also incubated under the same conditions. Single cysts and mixed sediment incubations were checked regularly for germination by stereomicroscope. After germination, individual cells were isolated by micropipette and incubated in separate petri-plates under the same culture conditions. When cells reached sufficient cell concentration, the clonal cultures were transferred to flasks containing 50ml of GSe medium and sub-cultured every 4 weeks thereafter. Duplicate flasks of each strain were also adapted to and held at 17°C ± 0.5°C at the same light intensity as above.

2.3. Encystment

At the time of isolation and incubation, the vast majority of *Scrippsiella* resting cysts showed no or poor calcification and exhibited no distinct morphological features. Encystment studies were carried out using the established laboratory cultures to aid the description of resting cyst morphology and variability, and assit species identification. One millilitre of ten cultured strains of *Scrippsiella* (SCBC17, SCBC18, SCBC19, SCPC21, SCPC23, SCPC36, SCPC39, SCPC51, SCPC73 and SCPC116) germinated from single cysts were inoculated into two petri dishes

containing 20ml of a nitrate/phosphate-deficient GSe medium. Petri dishes were sealed with Parafilm and incubated under the same conditions as previously described. Petri dishes were checked regularly for cyst production. Strains that did not produce resting cysts within two months were shifted to the culture room at a lower temperature (17°C ± 0.5°C) and examined regularly thereafter. After encystment, the resting cyst morphology was examined by both light and electron microscopy.

2.4. Microscopy

2.4.1. Light Microscopy

Cysts of *Scrippsiella* spp. in sediment samples and cultures were photographed with an Olympus BH-2 light microscope equipped with a Leica DC300F digital imaging system. After isolation but prior to incubation, cysts were photographed with a Zeiss inverted microscope equipped with a Kodak DC120 digital camera. After germination, cell morphology was examined by light microscopy and photographed. Thecal plate patterns were visualised by staining with Calcofluor M2R (Fritz & Triemer 1985) and epifluorescence microscopy using a broadband UV light and Olympus filter set no. 488.

2.4.2. Scanning Electron Microscopy (SEM)

Two methods were utilised for SEM of *Scrippsiella* cells. In the first method, the cells were prepared using the cell stripping technique of Mason *et al.* (2003). In this method, 10ml of mid-logarithmic growth-phase cultures of each strain was briefly placed in 45ml centrifuge tubes, with the aim of stressing the cells as little as possible. The samples were mixed with 10ml of 15% Triton X-100 and incubated in a sonicating water bath (Branson 1200) at 29°C for 1hr and then centrifuged (1500 rpm) for 5 min. The concentrated cells were then diluted in 40% GSe medium (diluted in seawater) and left at room temperature for 30 min. The cells were centrifuged for another 5 min. and 2ml of concentrated cells mixed with 2ml of 10% glutaraldehyde and centrifuged for 5 min. Fixed cells were concentrated by

centrifuge, placed on polylysine coated coverslips (Marchant & Thomas 1983), and dehydrated through a graded methanol series (10-100% in eight steps). Samples were critical-point dried (Balzers CPD 020, Germany) via liquid CO2, sputter coated with gold (Balzers SCP004 sputter coater) and examined using either Philips Quanta 600 or JEOL (JSM-840) scanning electron microscope (20 KeV accelerating voltage). In the second method, no fixative was used. Ten ml of nutrient deficient culture (including cysts and cells) was concentrated by centrifuge (5 min., 1000×g), collected on poly-lysine coated cover- slips and dehydrated in a graded methanol series (10-100%). The samples were then dried with hexamethyldisilazane (HMDS) in two steps of 15 min. each (Nation 1983).

Individual cysts from field samples in processed sediments were located by light microscopy, isolated by micropipette, washed twice in distilled water, placed onto 1μm Nucleopore filters, mounted on aluminium SEM stubs and air-dried. All stubs were sputter-coated with gold as described above and examined under a Philips Quanta 600 or JEOL (JSM-840) scanning electron microscope.

2.5. DNA Extraction and Polymerase Chain Reaction (PCR)

DNA was extracted from 30 strains of *Scrippsiella* spp. established from germinated resting cysts and sediment samples. Of these, 10 cultures were established from germination of single cysts and 20 were established from single cells isolated from mixed sediment incubations. Approximately 10ml of the exponential growth phase of cultures was pelleted by gentle centrifugation (1500g for 5 min.), and the supernatant discarded. DNA was extracted using a phenol: chloroform: isoamyl alcohol gentle-lysis method (Bolch *et al.* (1998). The extracted DNA was precipitated by the addition of 3M sodium acetate and 2 volumes of cold ethanol, washed in 70% ethanol, air dried and resuspended in sterile MilliQ water or TE buffer. DNA quality and quantity was verified by electrophoresis through 1% agarose/TBE gels and comparisons to DNA size standards. Sub-samples of DNA were diluted to an approximate concentration of 10 ng μl^{-1} for PCR and used as template in PCR amplification.

The internal transcribed spacer region of the rRNA genes was amplified using the primers of Adachi *et al.* (1994): ITSA (5'- CCA AGC TTC TAG ATC GTA ACA AGG (ACT) TCC GTA GGT- 3') and ITSB (5' -CCT GCA GTC GAC A(TG) ATG CTT AA(AG) TTC AGC (AG)GG - 3'). PCR reactions were performed in 50µl volumes in thin-walled 200µl PCR tubes. The PCR contained, Bioline NH_4 PCR reaction buffer (160mM $(NH_4)_2SO_4$, 670mM Tris-HCL and 0.1% Tween-20), 3mM $MgCl_2$, 200µM dNTPs , 10pM of each Primer, 1U BioTaq DNA polymerase (Bioline, UK) and 10 ng of template DNA. Thermocycling was as follows: initial denaturation of 2 min. at 94°C, followed by 35 cycles of 94°C for 1 min., annealing at 60°C for 1 min., elongation at 72°C for 2 min.; and a final extension of 6 min. at 72°C. PCR products were stored at -20°C until further analysis.

Five microliters of each completed PCR reaction was verified for the presence of the specific amplification product by electrophoresis through 1% agarose/TBE gels. Successful PCR products were purified using Montage PCR Devices (Millipore, USA) according to the manufacturer's protocols and the purified product quantified by comparison to DNA standards of known concentration by gel-electrophoresis (as above and using a Turner TBS-380 DNA fluorometer following the manufacturer's instructions.

2.5.1. DNA Sequencing

Purified PCR products were used as template for DNA sequence reactions. The cycle sequencing reactions used a Dye Terminator Sequencing Kit (Beckman-Coulter, Fullerton, CA, USA) following the manufacturer's protocols, but using 40-60 ng of DNA and 3.2pmol of primer per 10µl reaction, and the standard thermocycling program. PCR products were sequenced in both directions using each amplification primer in separate forward or reverse sequence reactions. Sequence reactions of rDNA-ITS products were electrophoresed on Beckman-Coulter CEQ2000 capillary sequencer at the University of Tasmania, Central Science Laboratory (Hobart).

2.5.2. Alignment and Phylogenetic Analyses

Nucleotide sequences were checked by manual inspection of electropherograms in both directions, and base-calling errors corrected manually using the BioEdit software (Hall 1999). Sequences obtained from the present study and related dinoflagellate sequences available from GenBank were aligned using ClustalX version 1.83 (Jeanmougin *et al.* 1998), and the alignments improved by manual inspection. The nucleotide alignment and PCR gel electrophoresis data are shown in Appendices 2 and 3. Table 1 shows the taxa included in the alignment and their GenBank accession numbers.

The computer program PAUP* 4.0b10 for Macintosh (PPC) (Swofford 2002) was used for all phylogenetic analyses. The dataset contained 53 taxa, and 757 characters (including gaps introduced into the alignment). The related peridinioid taxa, *Heterocapsa pygmaea* A.R. Loeblich, *Heterocapsa triquetra* Ehrenberg(Stein), *and Peridinium cinctum* Muller were used as outgroup species. Significant phylogenetic structure in the datasets was estimated by the random tree method and probability tables using the critical values of g_1 (Hillis & Huelsenbeck 1992). Phylogenies were constructed using neighbour-joining (NJ) and maximum parsimony (MP). Neighbour-joining analyses used the mean distance metric. Searches for the most parsimonious tree using the branch and bound algorithm proved to be unreasonably time consuming (weeks) with the large dataset, therefore the heuristic search algorithm was used to find a range of candidate most parsimonious trees. All characters were equally weighted and gaps were treated as missing data, with multistate characters (DNA ambiguities) interpreted as uncertainty. Support for branching phyogenetic trees was estimated using bootstrap analyses (Felsenstein 1985) to generate 100 replicates in both NJ and MP trees. Bootstrapping of parsimony anlayses proved unfeasible with the 57 taxa dataset, therefore the dataset was reduced to 24 taxa, with one to two taxa included to represent each terminal cluster identified from the parsimony analysis using 57 taxa.

Table 1: List of taxa and strains and their GenBank accession used in this study

Taxon	Strain No.	GenBank No.
Scrippsiella precaria	CS-294	AY499518
Scrippsiella ramonii	SZN7	AF527820
Scrippsiella infula	GeoB 110	AY499523
Scrippsiella rotunda	SZN66	AF527821
Scrippsiella trifida	GeoB 109	AY499521
Scrippsiella lachrymosa	D192	AY728078
Scrippsiella sp.	CS-168	AY499533
Scrippsiella sp.	GeoB*160	AY499526
Scrippsiella sp.	D1006	AY728079
Scrippsiella sp.	M34-*25/5	AY499531
Scrippsiella sp.	GeoB 188	AY499524
Scrippsiella sp.	GeoB 138	AY499525
S. trochoidea var. *aciculifera*	SZN63 clone 135	AF527078
S. trochoidea var. *aciculifera*	GeoB 228	AY499529
S. trochoidea var. *aciculifera*	GeoB*213	AY676164
S. trochoidea var. *aciculifera*	SCCAP499	AF527066
S. trochoidea var. *aciculifera*	SZN60	AF527071
Scrippsiella trochoidea	NIES 369	AY499530
Scrippsiella trochoidea	GeoB*200	AY676157
Scrippsiella trochoidea	D201	AY728080
Scrippsiella trochoidea	IO 26-01	AY676163
Scrippsiella trochoidea	GeoB*201	AY676158
Scrippsiella trochoidea	GeoB*214	AY676160
Scrippsiella trochoidea	SZN82 clone 49	AF527101
Scrippsiella trochoidea	SZN33	AF527070
Scrippsiella trochoidea	SZN61	AF527075
Scrippsiella trochoidea	SZN64	AF527079
Calciodinellum sp.	GeoB 120	AY676148
Calciodinellum albatrosianum	GeoB 149	AY676143
Calciodinellum albatrosianum	M34-17	AY676144
Calciodinellum albatrosianum	M34-*26/4	AY676145
Scrippsiella lachrymosa	IO 25-01	AY676150
Scrippsiella sp.	GeoB*161	AY499527
Scrippsiella trochoidea	IO 14-01	AY676162
Scrippsiella sweeneyae	NIES 684	AY499520
Pentapharsodinium dalei	SZN19	AF527817
Calciodinellum operosum	CalopeD006	AY327462
Calciodinellum levantinum	GeoB 122	AY676146
Calciodinellum levantinum	GeoB*165	AY676147
Ensiculifera aff. *imariensis*	D207	AY728076
Ensiculifera cf. *imariensis*	JB3	AF527814
Ensiculifera loeblichii	UTEXLB1595	AF527815
Scrippsiella hangoei	SHTV1	AY499515
Pernambugia tuberosa	GeoB 61	AY499519
Heterocapsa pygmaea	CCMP1322	AB084093
Heterocapsa triquetra	NIES 7	AB084101
Peridinium cinctum	CCAC 0102	AY499511
Scrippsiella irregularis	SCBC17	Present study
Scrippsiella irregularis	SCBC19	Present study
Scrippsiella sp. 1	SCPC21	Present study
Scrippsiella sp. 2	SCPC116	Present study

Scrippsiella trochoidea	SCBC18	Present study
Scrippsiella trochoidea	SCPC36	Present study
Scrippsiella trochoidea	SCPC39	Present study
Scrippsiella trochoidea	SCPC51	Present study
Scrippsiella trochoidea	SCPC73	Present study

3. Results

3.1. Morphology

A total of 20 clonal cultures were established from the nine sites. Ten strains established from germination of single cysts were examined in detail. The remaining strains (i.e. cloned successfully from individual cells) appeared morphologically and genetically similar to strains referred to herein as *Scrippsiella* sp. 2. The motile cells of the remaining 10 strains could be separated into four distinct morphotypes: *Scrippsiella irregularis* sp. nov. (SCBC17, SCBC19), *Scrippsiella* sp. 1 (SCPC21, SCPC23), *Scrippsiella* sp. 2 (SCPC116), and *Scrippsiella trochoidea* (SCBC18, SCPC36, SCPC39, SCPC51, SCPC73). All vegetative cells displayed typical *Scrippsiella* plate formula: Po, X, 4', 3a, 7", 6C (5C+1 transitional plate= T-plate), 5S, 5"', 2"", with the exception of strain SCPC116 where a small proportion of cells exhibited and additional postcingular plate (6"').

Despite the consistency of overall tabulation, the cell shape and the shape of various plates showed clear variation among the four morphotypes. The arrangement and shape of plates, overall body shape, and size in the vegetative cell and also the cyst morphology all showed consistent variation and are used to separate the *Scrippsiella* morphotypes described here. The arrangement of the intercalary plates in relation to the apical and precingular plates is an important feature of peridinioid species. The arrangement may be symmetrical with 3', 2a and 4" in a row vertically (bipesioid tabulation; e.g. *Scrippsiella trochoidea)*; or asymmetrical (cinctioid tabulation; e.g. *Scrippsiella precaria* Montresor & Zingone) where the 3', 2a, and 4" plates are not arranged vertically (Fensome *et al.* 1993).

In the following section, the motile cells and cysts of four different morphospecies, from southeast coast of Iran are described and the results are summarised in Table 2.

Table 2: Comparison of the morphological features of the *Scrippsiella* species described in this study

Motile cell	***S. irregularis***	***Scrippsiella* sp. 1**	***Scrippsiella* sp. 2**	***Scrippsiella trochoidea***
Length (μm)	17-29	19-30	22-29	18-34
Width (μm)	13-23	15-25	20-25	17-29
Epitheca	Conical-rounded	Conical	Conical-rounded	Conical
Hypotheca	Rounded	Rounded	Rounded	Rounded
1' plate	Wide	Medium	Narrow	Medium
1` plate shape	Asymmetrical rhomboidal	Asymmetrical rhomboidal	Rhomboidal (anterior part) rectangular (posterior part)	Asymmetrical rhomboidal
Dorsal epithecal tabulation	Cinctioid	Bipesioid	Bipesioid	Bipesioid
Postcingular plates	5'''	5'''	6'''(5''')	5'''
Plate ornamentation	Simple pores	Simple pores	Simple pores	Simple pores
Colour	Brownish-green	Dark brown	Light yellow to green	Golden-brown
Nucleus shape	Spherical	Spherical	Sub-spherical	Spherical
Nucleus position	Hypotheca	Central-left	Hypotheca	Central
Resting cyst				
Shape	Spherical to oval	Spherical to sub-spherical	Spherical	Spherical to oval
Size	20-26μm diameter	27-31μm	39-64μm	18-37μm
Colour	Grey	Dark brown to green	Light brown	Dark brown
Ornamentation	Numerous pointed or capitate spines	Pointed spines or polygonal crystal	Close-packed crystalline network	Three cornered spines with irregular base plate

3.1.1. Species Description

***Scrippsiella irregularis* Attaran-Fariman & Bolch sp. nov.** **Figs 1-19**

Diagnosis:

Cellulae ovoideae, leviter dorsiventraliter compressae, 17-29μm longae, 13-23μm latae. Epitheca rotundata, conica lateribus convexis, sine cornu apicali; hypotheca rotundata. Cingulum subaequatoriale descendens, per dimidiam partem vel per totum latitudinis suae dislocatum. Formula laminarum Po, X, 4' 3a, 7", 6C (5C+1), 6S, 5"', 2"". Laminae 1a et 3a adjacentes, hexagonae et forma similes. Lamina 2a infra et inter laminas 1a et 3a, subrectangularis vel rotundata. Sulcus valde distinctus, antapicem non attingens. Lamina anterior sulcalis (Sa) latior quam longior, super porum flagellarum et laminas sulcales extensa. Nucleus magnus sphaericus, in hypotheca. Chloroplasti aliquot, globulares vel taeniiformes, peripherales, fuscovirides. Cystae quiescentes sphaericae vel ovoideae, 20-26μm in diametro, spinis multis calcareis acicularibus vel capitatis 3.5-4.5μm longis.

Cells ovoid in outline, slightly dorso-ventrally compressed, 17-29μm long, 13-23μm wide. Epitheca rounded, conical with convex sides and no apical horn; hypotheca rounded. Cingulum sub-equatorial and descending, displaced 1/2 to 1 cingulum width, narrowing dorsally. Plate formula Po, X, 4' 3a, 7", 6C (5C+1), 6S, 5"', 2"". Plate 1a and 3a adjacent, hexagonal and similar in shape. Plate 2a below and between plates 1a and 3a, sub-rectangular to rounded in shape. Sulcus well defined, not extending to the antapex. Anterior sulcal plate (Sa) broader than long, extending over flagella pore and sulcal plates. Nucleus large, spherical and positioned in the hypotheca. Cells contain several globular to ribbon-like, peripherally placed, dark brown-green chloroplasts. Resting cysts are spherical to ovoid, 20-26μm diameter, with numerous non-tabular calcareous acicular or capitate spines 3.5-4.5μm in length.

***HOLOTYPE*:** Figure 1; Strain SCBC17

An individual cyst was isolated, germinated and cultured from coastal sediments collected from Bahoo-Kalat estuary on the southeast coast of Iran in March 2004. Cultures are kept in the University of Tasmania, School of Aquaculture, Algae Culture Unit, Launceston, Australia.

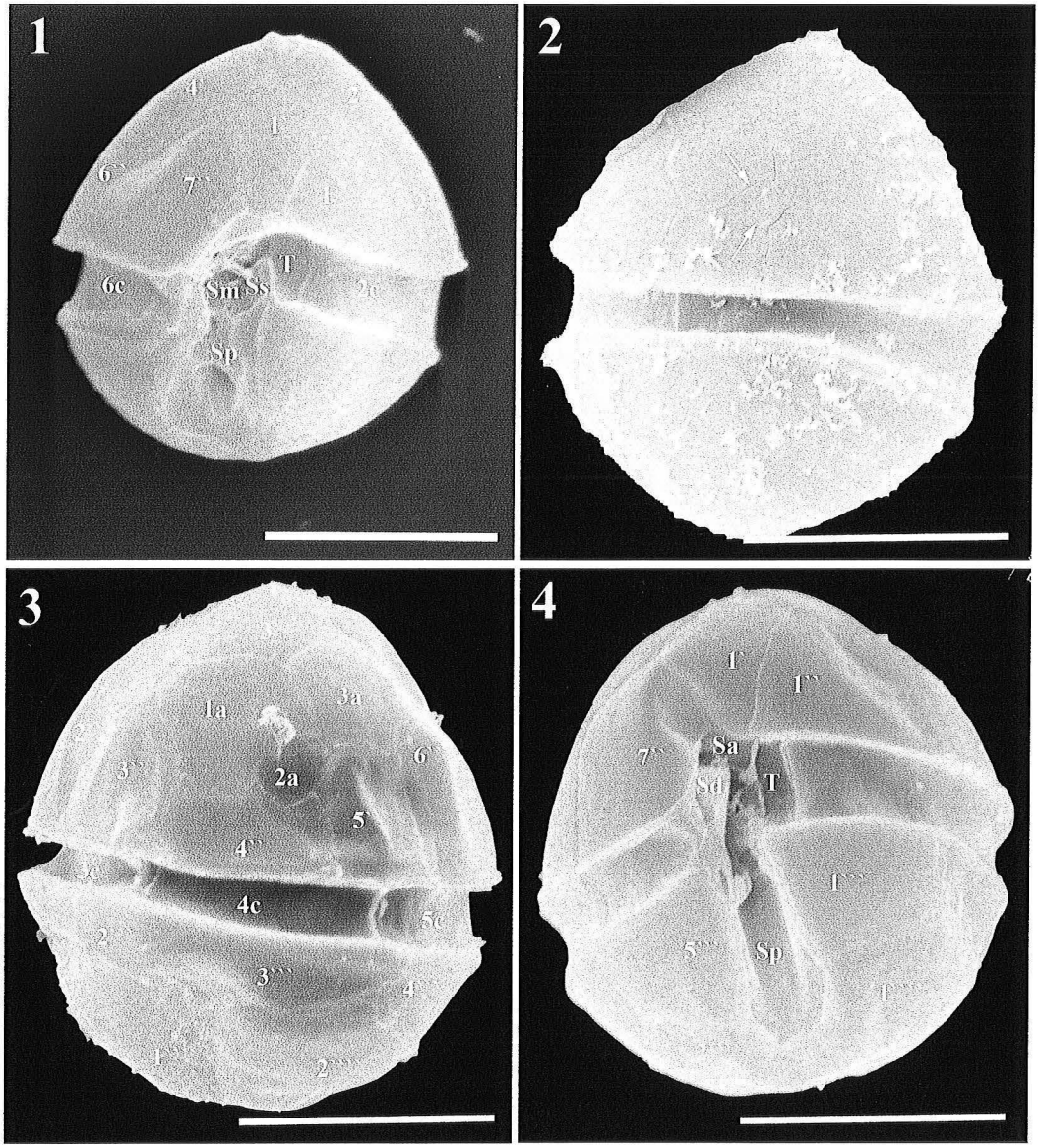
TYPE LOCALITY: Bahoo-Kalat Estuary, Iran.

ETYMOLOGY: *irregularis*; in which symmetry is destroyed by some inequality of parts; referring to the lack of symmetry of intercalary plates.

DISTRIBUTION: Southern coast of Iran, Bahoo-Kalat Estuary.

The vegetative cells of cultures SCBC17 and SCBC19 are ovoid slightly dorso-ventrally compressed and possessing a rounded epitheca with convex sides. The apex is rounded with no pronounced apical horn (Figs 1-4). The hypotheca is semi-circular in outline. The cells are 17-29µm (mean=22µm, n=50) in length and 13-23µm in width (mean=18µm, n=50). The epitheca is longer than the hypotheca with a ratio of 1.4 in length. The width of the cingulum varies from 2.6-3.6µm (mean=2.9µm, n=20), and is left handed and displaced one-half to one (1/2 -1) cingulum width (Figs 1, 4 & 10). The border between the cingulum and hypotheca is curved and the cingulum appears to be narrower on the dorsal side (Figs 2 & 12). The plate formula is the same as other *Scrippsiella* species (i.e. Po, X, 4', 3a, 7", 6C (5C+1 transitional plate=Tplate), 5S, 5"', 2"" see Figs 1, 3, 4 & 6), but the shape and position of the three intercalary plates are distinct. The plates are diagonally located on the dorsal part of the epicone (Figs 2 & 3). Plates 1a and 3a are six sided and in contact on the anterior side (Fig. 3). Plate 2a is sub-rectangular with two convex sides and positioned between the posterior sides of plates 1a and 3a (Figs 2, 3 & 9).

The sulcus is composed of five plates: S_a, S_p, S_s, S_d and S_m plates (Figs 1 & 4). S_a is located between 1' and 7" and shows a hook-like extension over the flagella pore and the sulcal plates and border with the T-plate (Figs 4, 15 & 14). The S_p plate is extended into the hypotheca without touching the antapex (Fig. 4). The S_s plate is small and rhomboidal in shape and connected to the T plate on one side (Figs 1 & 8). The S_d plate is triangular and positioned beneath the S_a plate, touching the 7", 6C, 5"' and S_p plates (Figs 4 & 5). The S_m plate is almost completely hidden by the anterior part (wing) of the S_p plate (Fig. 4) and can be seen when the S_d plate is damaged (Fig. 8). The first cingulum plate (T-plate) is small in size and borders the S_a and S_s plates (Figs 8, 14 & 15).



Figs 1-4. SEM. Vegetative cell of *Scrippsiella irregularis.*

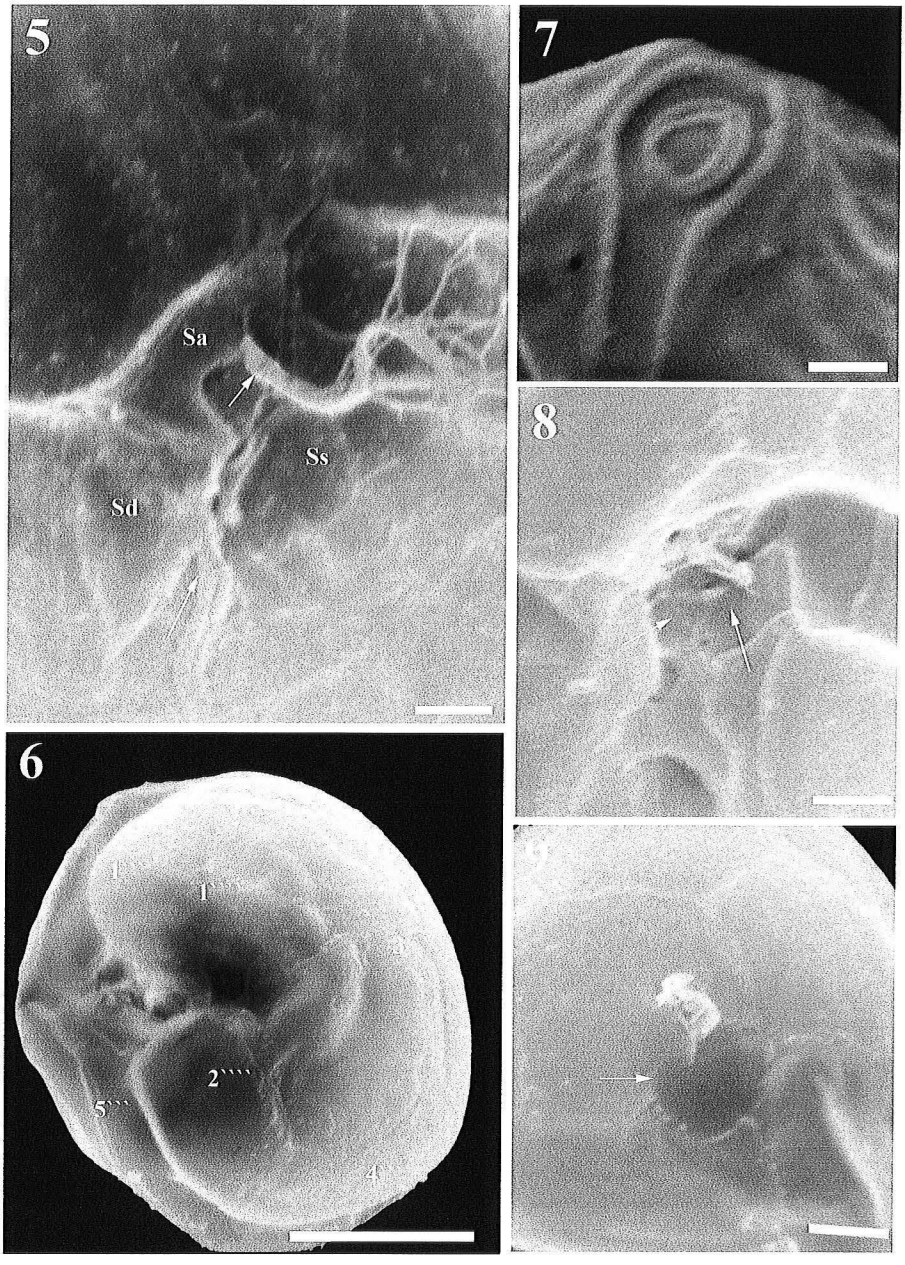
Fig. 1. Ventral view showing the plate pattern, note the S_m plate.

Fig. 2. Dorsal view showing the second intercalary plate (arrows). Note the cingulum wing on the dorsal side of the cell.

Fig. 3. Dorsal view of cell showing the plate pattern. Note the rounded shape and size of 2a plate.

Fig. 4. Ventral view showing sulcal and post cingular (1''', 5''') plates, note S_m plate almost hidden by wing of S_d plate.

All scale bars= 10μm.



Figs 5-9. SEM. Theca cell of *Scrippsiella irregularis.*

Fig. 5. Sulcal area, showing transverse and longitudinal flagellate, note the shape of S_a and S_d plates.

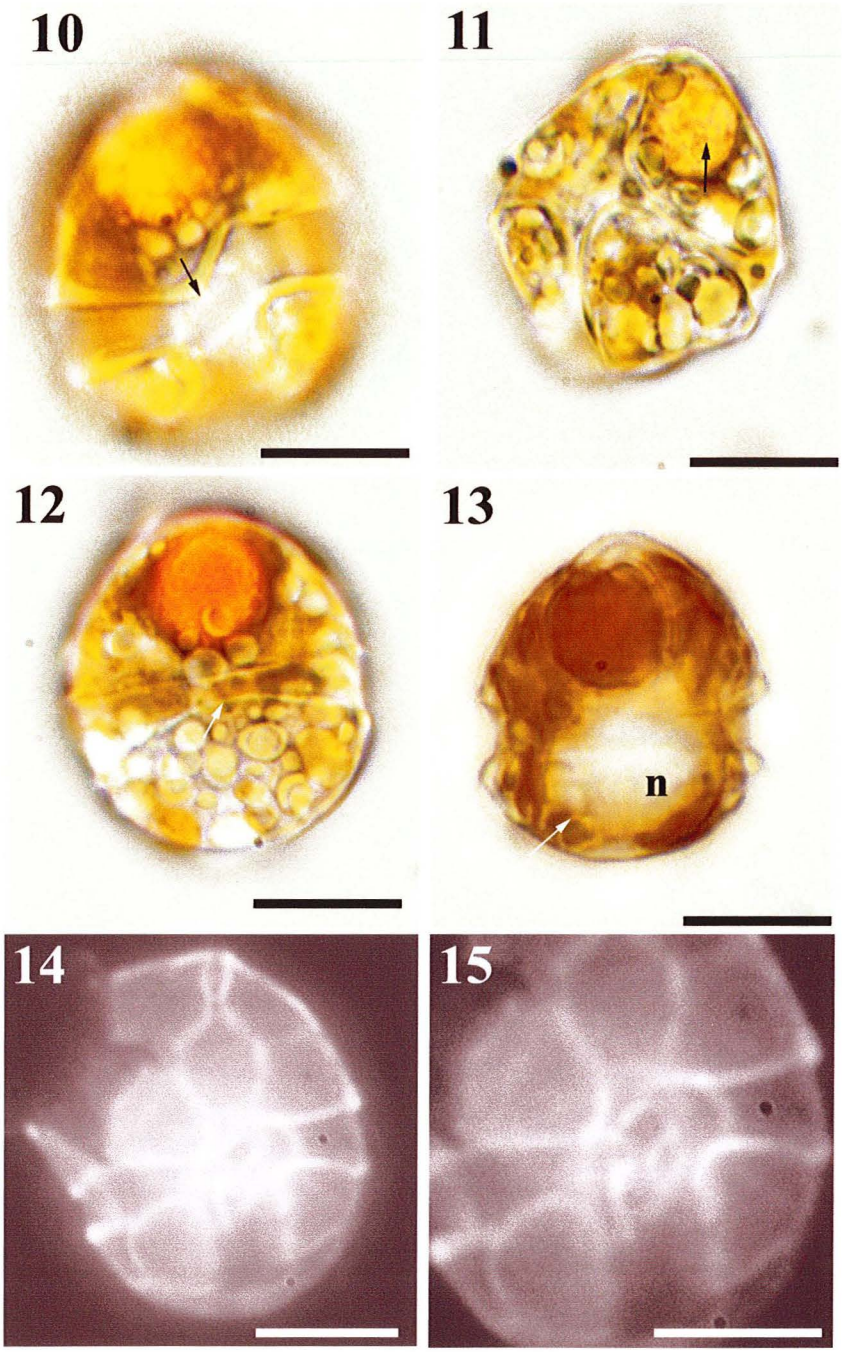
Fig. 6. Antapical view.

Fig. 7. Apical view showing apical pore and canal plates.

Fig. 8. Sulcal plates, showing the shape of S_m (left arrow) and S_s plates (right arrow). Note the shape of the transitional plate.

Fig. 9. Dorsal view, showing intercalary plates, note 2a plate (arrow).

All scale bars=2µm, except Fig. 6=10µm.



Figs 10-15. LM. *Scrippsiella irregularis.*

Fig. 10. Ventral view of cell showing cingulum displacement (arrow).

Fig. 11. Ventral view. Arrow showing large accumulation body.

Fig. 12. Dorsal view. Note cingulum narrowing on dorsal side (arrow).

Fig. 13. Dorsal view of cell showing the large nucleus (n) in the hypotheca. Note irregular arrangement and shape of the chloroplasts (arrow).

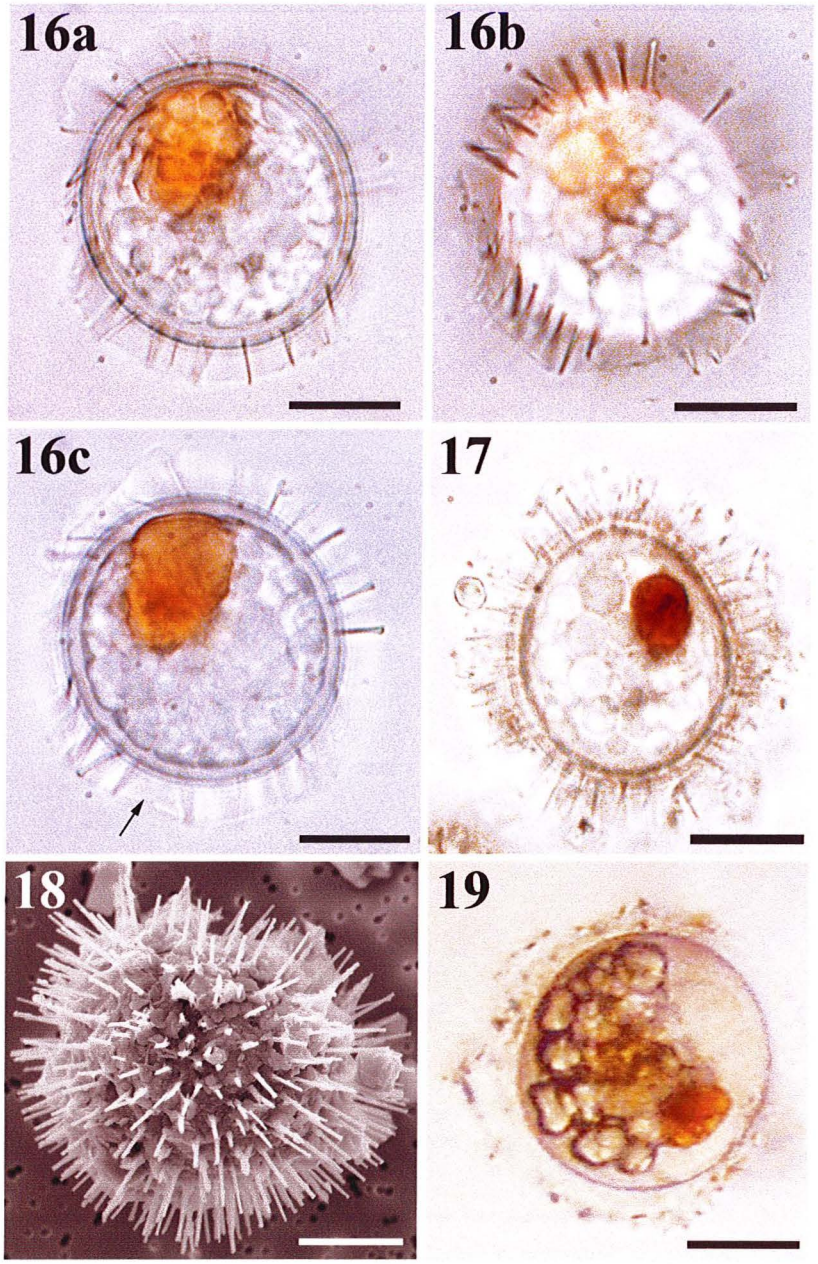
Figs 14-15. Fluorescence images of a calcofluor-stained cell, showing details of ventral plate patterns. Note the T-plate does not extend toward the first apical plate.

All scale bars=10μm.

The large spherical nucleus is positioned in the hypotheca (Fig. 13). A large yellowish-orange accumulation body is visible in the epitheca, near the apex (Figs 10-13). Cells contain numerous peripherally placed brown-green chloroplasts that are round to oval in shape with irregular arrangement (Figs 12 & 13). A few small pores are scattered on the cells surface (Fig. 5).

The cells grow quickly in laboratory culture. In nitrate and phosphate deficient media only a few cells encysted and even a shift in temperature from 27°C to 17°C did not increase the rate of encystment. No encystment was seen in nutrient-replete cultures.

Cultured resting cysts are covered with numerous, long, non-tabular calcareous spine with both capitate and pointed termination. A clear thin outer membrane surrounded the spines of the cysts produced in nutrient-deficient medium (Figs 16a-c). The cyst body is spherical to oval in shape and the cysts appear light grey to brown in colour with a red-orange accumulation body. The size of the cyst body varies from 20-26μm (mean=24μm, n=15) in diameter, with the process length ranging from 3.5-4.5μm (average=4μm, n=15). No clearly defined archeopyle was noted in cultured or wild resting cysts. Similar calcareous cysts were also isolated from marine sediment (Figs 17 & 18) but both *S. irregularis* cultures established in this study were germinated from clear-walled spineless cysts (Fig. 19). While cultured cysts possess both pointed and capitate spines, the wild cysts attributed to this species possess predominantly pointed processes.



Figs. 16-19. Resting cyst of *Scrippsiella irregularis.*

Fig. 16a. LM. Cultured resting cyst showing cell content and accumulation body.

Fig. 16b. Surface focus showing the spines.

Fig. 16c. Transparent membrane surrounding the spines (arrow).

Fig. 17. LM. Resting cyst isolated from sediment.

Fig. 18. SEM. Wild cyst showing the calcareous spines.

Fig. 19. LM. Original cyst that established the culture. Note the lack of spines.

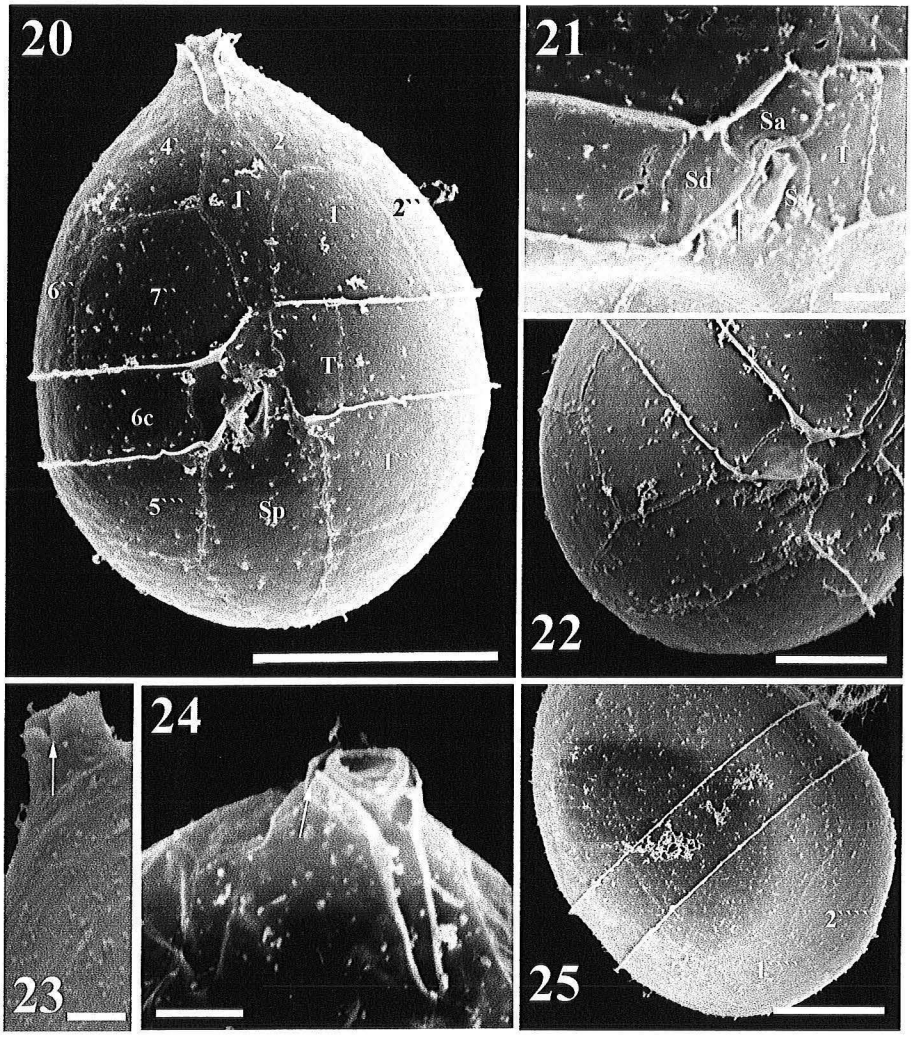
All scale bars=10μm.

3.1.2. Scrippsiella sp. 1 (Figs 20-39)

The vegetative cells of strains SCPC21 and SCPC23 possess a conical epitheca with convex sides and a round hypotheca (Fig. 32). The cell size ranges from 19-30μm in length (average=25μm, n=30) and 15-25μm in width (mean=20μm, n=30). The epitheca is longer than the hypotheca. The cingulum is left-handed with a displacement of less than half of its width (Fig. 20). The plate tabulation is typical of *Scrippsiella trochoidea*: Po, X, 4', 3a, 7", 5C+1 transitional plate, 5S, 5"', 2"" (Figs 20-29). However, the apical horn is clearly different from *Scrippsiella trochoidea.* The apical horn is pronounced and consists of a pore plate with a triangular ridge surrounded by a high collar with a V-shaped split in its right dorso-lateral side (Figs. 23 & 24). The cells have a long and wide canal plate, connecting the pore plate to the wide and slightly asymmetrical first apical plate. The sulcus is composed of five plates. There is no spine on the anterior sulcal plate (Figs 21, 22, 33 & 34).

The nucleus is large and located in the centre of the cell slightly toward the hypotheca (Fig. 30). Cells contain numerous dark brown-green, radially arranged chloroplasts with an irregular shape, and a brownish green accumulation body (Fig. 31). The colour of the cultures is distinct dark-brownish green colour and grow quickly in culture.

Spontaneous encystment was observed in the nutrient-replete old cultures. Only a few cysts were noted in nutrient-depleted GSe medium. The laboratory produced cysts were covered with irregular polygonal rounded calcareous crystals (Figs. 35-37) that varied from densely packed to irregularly spaced with distinct gaps (Figs 35, 36b). Some cysts exhibited clear spines (Fig. 38). No archeopyle was observed. The cysts are spherical to oval, dark-brown to green in colour, with a dark yellow or orange accumulation body (Figs 33 & 37). Cyst diameter varied from 27-37μm (mean=29.2, n=15). Ovoid cysts varied from 27-31μm (mean=29.7μm, n=10) in width and 29-37μm in length (mean=32).



Figs. 20-25. SEM. *Scrippsiella* sp. 1. thecate cells prepared using the cell stripping and cell swelling technique (Mason *et al.* 2003).

Fig. 20. Ventral view, showing the plate tabulation.

Fig. 21. Detail of sulcal region. Note that the S_m plate completely hidden by S_d plate (arrow).

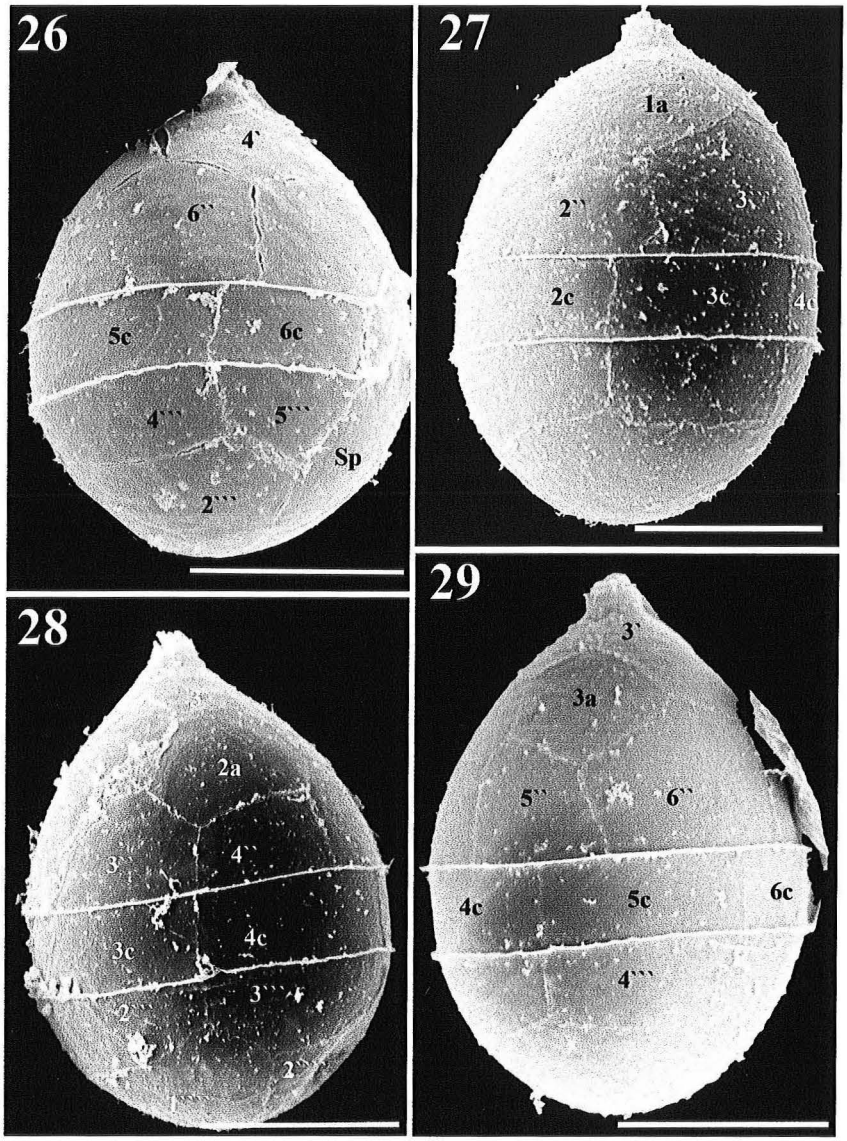
Fig. 22. Ventral view of cell showing the postcingular plates.

Fig. 23. Dorsal view of apical horn. Note a v-shaped notch (arrow).

Fig. 24. Ventral view showing apical pore and canal plate arrow head showing v-shaped split.

Fig. 25. Dorsal view showing antapical plates.

Figs 20, 22, 25 scale bar=10μm. Figs 21, 23, 24 scale bars=2μm.



Figs 26-29. SEM. *Scrippsiella* sp. 1 thecate cell prepared using the cell stripping swelling technique.

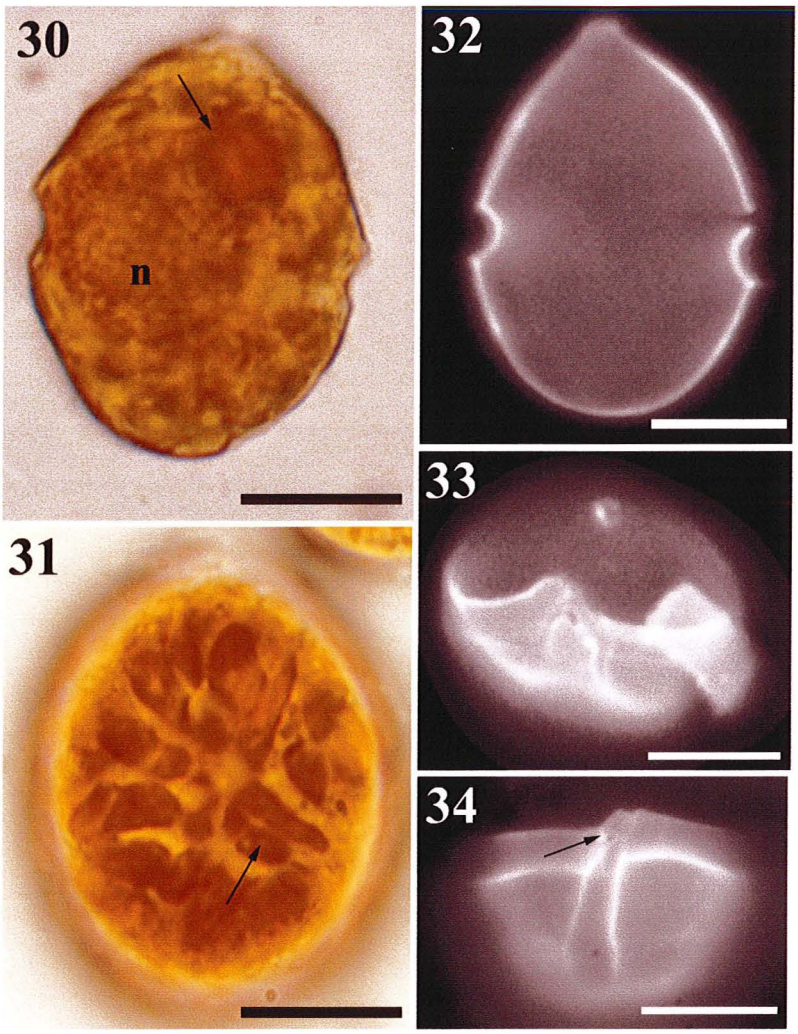
Fig. 26. Ventro-lateral view of left side of the cell showing the plates tabulation.

Fig. 27. Dorsal view showing the 1a and 3c complete plates.

Fig. 28. Dorsal view. Note 2a, 4", 4c, and 3"' plates are in vertical alignment.

Fig. 29. Latero-dorsal view, showing the 3a plate.

All scall bars=10μm.



Figs 30-34. LM. *Scrippsiella* sp. 1 thecate cell.

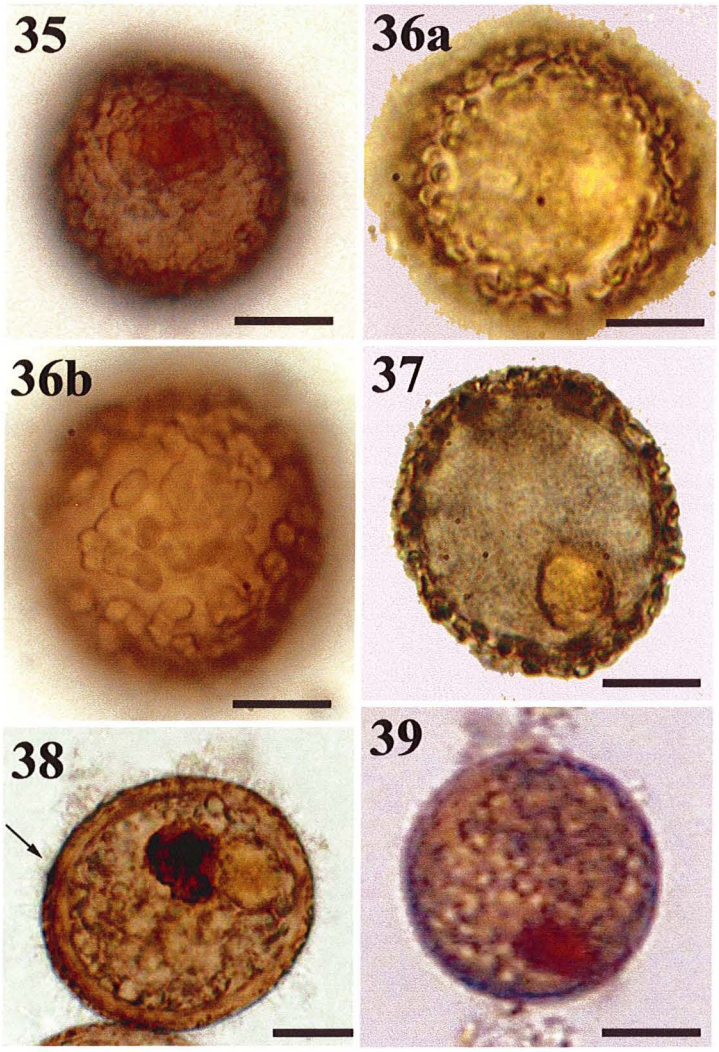
Fig. 30. Dorsal view of the cell, shows nucleus and accumulation body.

Fig. 31. Dorsal view, showing radially arranged chloroplasts.

Fig. 32. Fluorescence image of calcofluor stained theca showing cell outline.

Figs 33-34. Fluorescence image of calcofluor stained theca of broken cell showing lack of spine on S_a plate.

All scale bars are 10μm.



Figs 35-39. LM. *Scrippsiella* sp. 1 resting cysts.

Fig. 35. Cyst from culture showing densely packed calcareous ornamentation.

Fig. 36a. Cultured cyst showing crystalline ornamentation.

Fig. 36b. Surface focus of cyst, showing gaps between calcareous ornamentation.

Fig. 37. Deep focus showing the relative length of the calcareous ornamentation compared to the cyst diameter.

Fig. 38. Cultured cyst with short, clear spines (arrow).

Fig. 39. Original wild cyst that established the culture. Note lack of any ornamentation on the cyst wall.

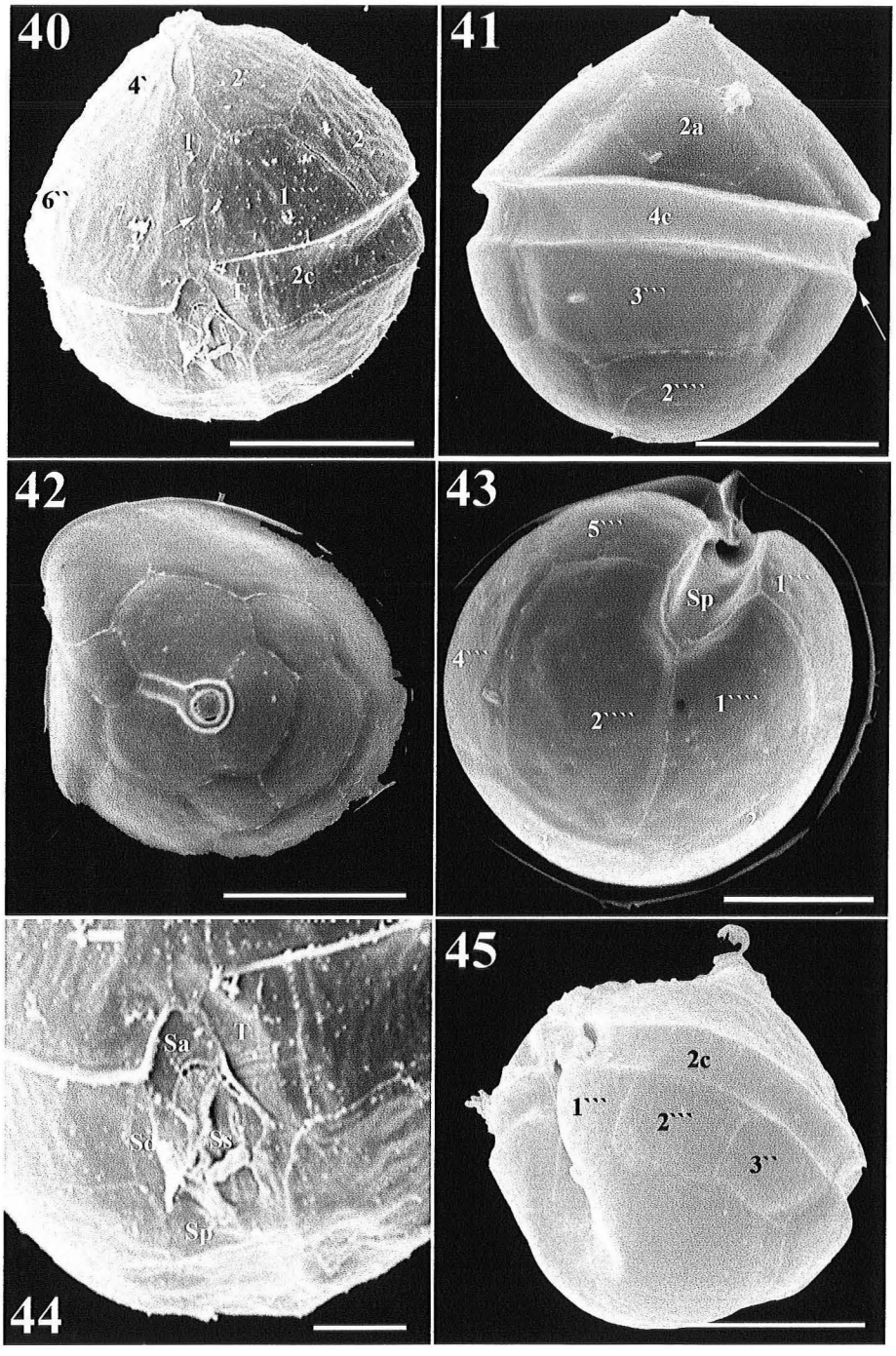
All scale bars= 10µm.

3.1.3. Scrippsiella sp. 2 (Figs 40-51)

The thecate cells of strain SCPC116 have a rounded-conical epitheca, and a rounded hypotheca (Figs 40-51). The epitheca and hypotheca are of similar size. The cell size varies from 22μm to 29 in length (average=25μm, n=15) and from 20-25μm in width (average=22μm, n=15). The cingulum is left-handed and descending with a displacement of 1/2-3/4 of its width. The anterior cingulum margin is more pronounced than the posterior margin. Many simple trichocyst pores are scattered on the cell wall.

The plate tabulation is Po, X, 4', 3a, 7'', 6C (5C+1 transitional plate=T plate), 5S, 5''' (6'''), 2''''). There is variation in number of plates, with most cells showing an extra postcingular plate between plates 2''' and 3''' (Figs 45 & 47). The first apical plate is differed in shape from the other four *Scrippsiella* species described here. The plate consists of two parts; the anterior part that is wide and symmetrical rhomboid in shape; the posterior part which is narrow and rectangular in shape (Fig. 40). This plate contacts both the transitional and anterior sulcal plates. The apical horn is well defined (Fig. 40). The apical pore plate possesses a circular ridge and the canal plate is long (Fig. 42). The sulcus is narrow and well defined (Fig. 43).

The large nucleus is sub-spherical and positioned in the centre left of the cell (Figs 49 & 50). The cells contain one (or few) light-yellow to green, elongated, highly branched and peripherally-placed chloroplast/s (Fig. 51). A clear accumulation body is present in the epitheca. Compared to the other species in this study, this culture grows slowly and reaches low maximum cell concentrations. No encystment was observed in stationary-phase cultures, however, a low concentration of cysts was produced in nutrient-deficient GSe medium.



Figs. 40-45. SEM. *Scrippsiella* sp. 2. thecate cells. The cell stripping and swelling technique was used. for Figs. 40 and 42.

Fig. 40. Ventral view of the cell. Note the narrow first apical plate.

Fig. 41. Dorsal view of the cell, showing the hexagonal 2a plate. Note pronounced cingular margin in eitheca and not well defined in posterior edge (arrow).

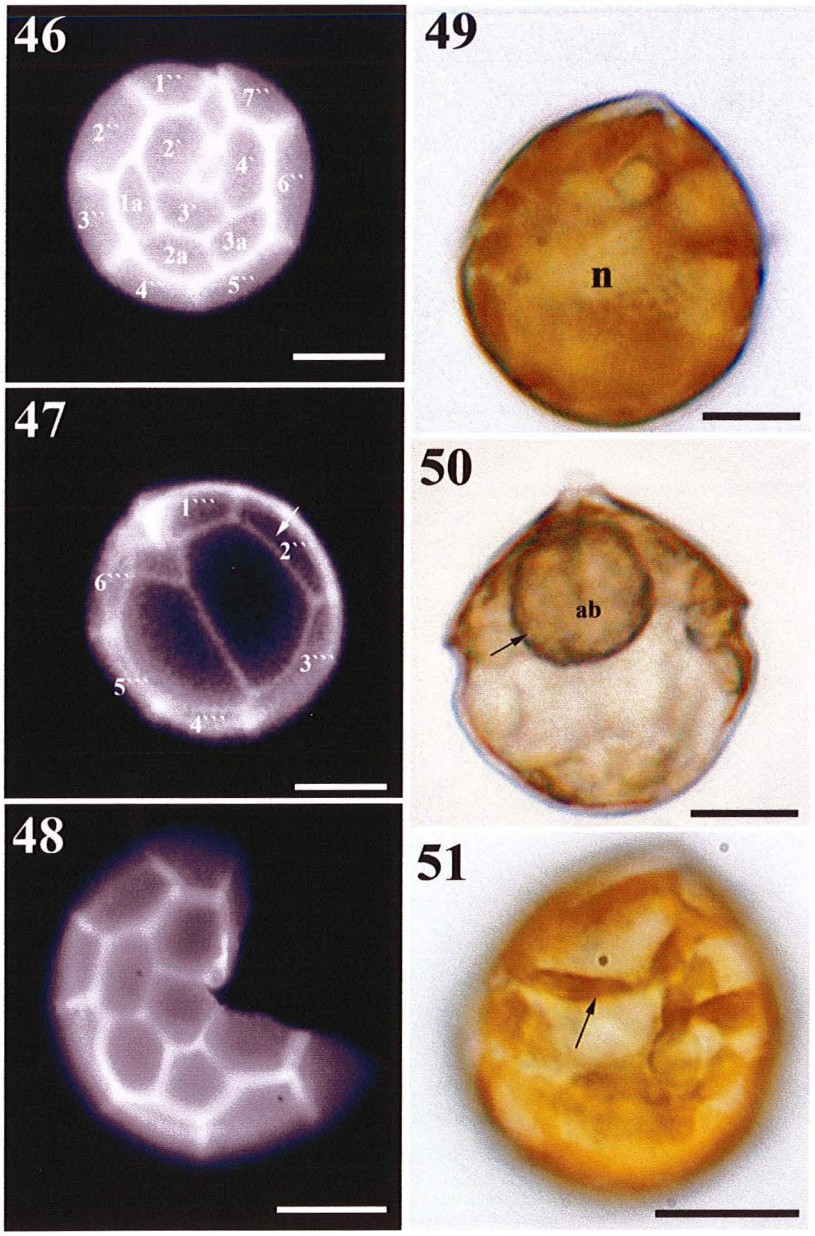
Fig. 42. Apical view showing epithecal tabulation.

Fig. 43. Antapical view showing antapical plate and 5 postcingular plates.

Fig. 44. Detail of sulcal plates.

Fig. 45. Latero-antapical view. Note the extra postcingular plate between 2``` and 3```.

All scale bars=10μm, except for Fig. 44 scale bar=5μm.



Figs 46-51. LM. *Scrippsiella* sp. 2 thecate cells.

Fig. 46. Calcofluor stained cells. Apical view of the cell showing epithecal plate pattern.

Fig. 47. Calcofluor stained cells. Antapical view, cell with 6''' plate.

Fig. 48. Calcofluor stained cells. Apical view.

Fig. 49. Dorsal view showing the nucleus.

Fig. 50. Dorsal view. Note large accumulation body (arrow) and nucleus.

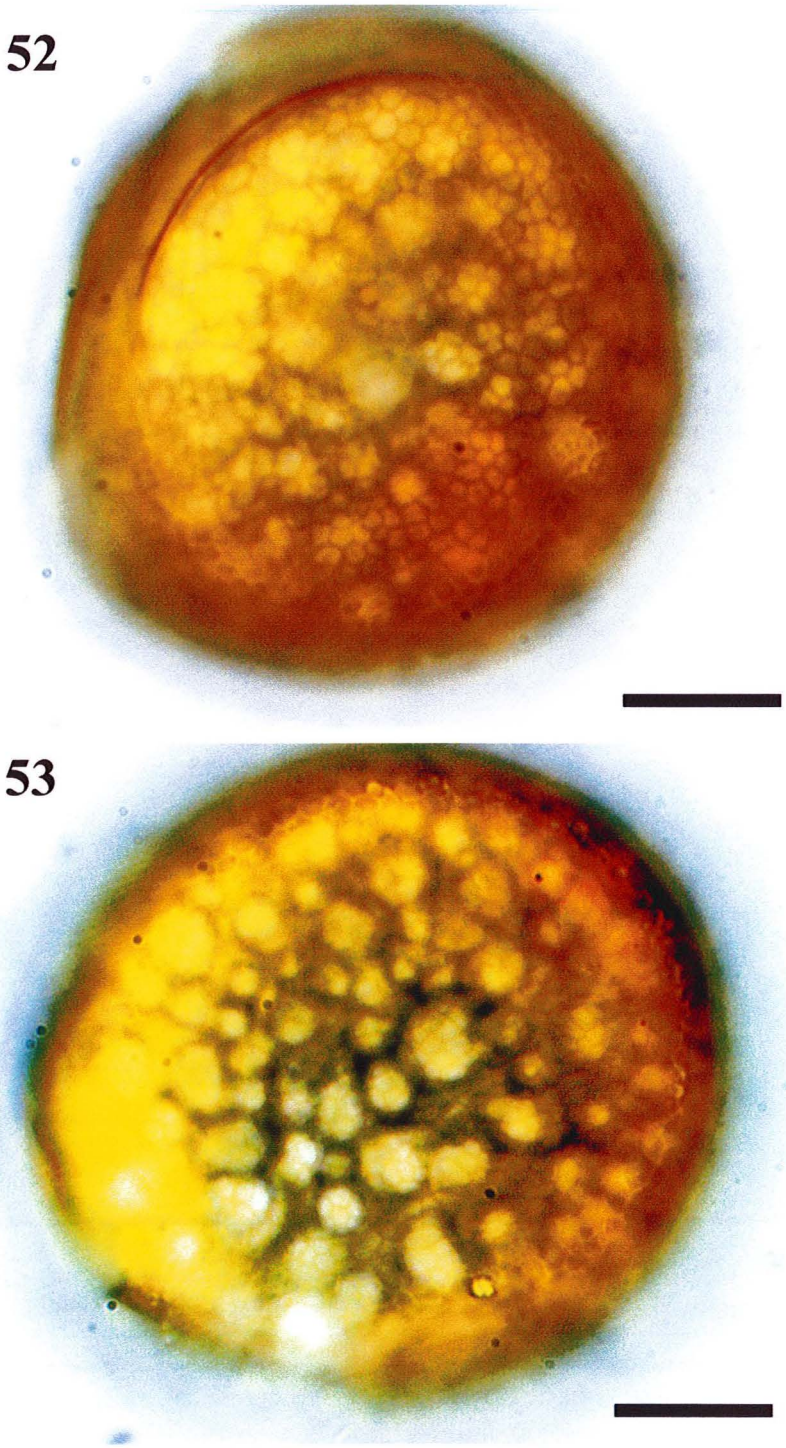
Fig. 51. Dorsal view. Note peripheral branched chloroplast/s (arrow).

All scale bars=10μm.

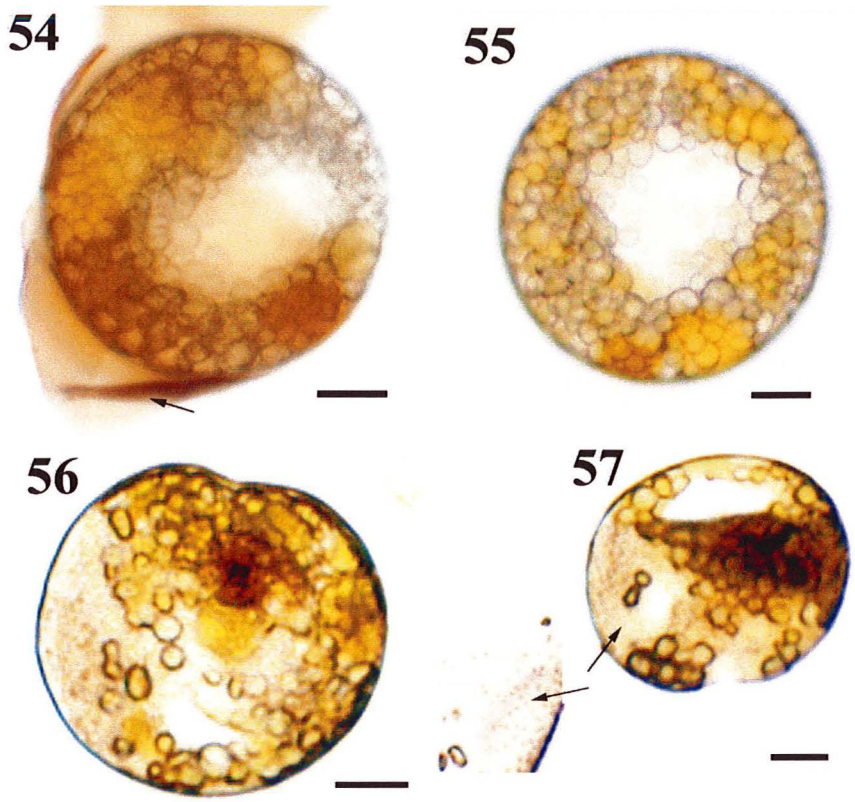
The cysts of *Scrippsiella* sp. 2 are large and vary from 39-64μm (mean=49μm, n=10) in diameter. Wild cysts germinated to produce cultures were spherical without spines or clear ornamentation, however, cultured cysts are spherical to slightly sub-spherical in shape. The wall is transparent, light-brown and covered with densely packed crystals with 3-6 sides that form a reticulate (Figs 52 & 53) pattern reminiscent of the microreticulate cyst-forming species of *Gymnodinium* (Chapter 3). The surface ornamentation appears flat by light microscopy and no paratabulation is evident. The calcareous ornamentation could be dissolved in hydrochloric acid, leaving only the inner organic cyst body.

The calcareous outer cyst wall is quite fragile and, by slightly pressing the coverslip, it is easily separated from the inner organic wall (Fig. 54). The cyst contents are globular and green in colour with a dark yellow accumulation body. Some other yellow contents, similar to an accumulation body, appear in the cyst but are poorly defined (Fig. 55). Despite careful SEM preparation, SEM of the cyst remains unsuccessful due to the limited number of cysts available.

Subsequently, similar cysts with a network crystal structure on the outer wall were found in wild sediment that were slightly different in shape. Wild cysts were sub-spherical to slightly reniform (Figs 56 & 57). The size ranges from 33-65μm in length (mean=55μm, n=9) to 30-59μm in width (average=48μm, n=9) with the length/width mean ratio=1.2.



Figs 52-53. LM. Resting cyst of *Scrippsiella* sp. 2 from culture. Cyst showing calcitic reticulate structures on the outer wall.
Scale bars=20µm.



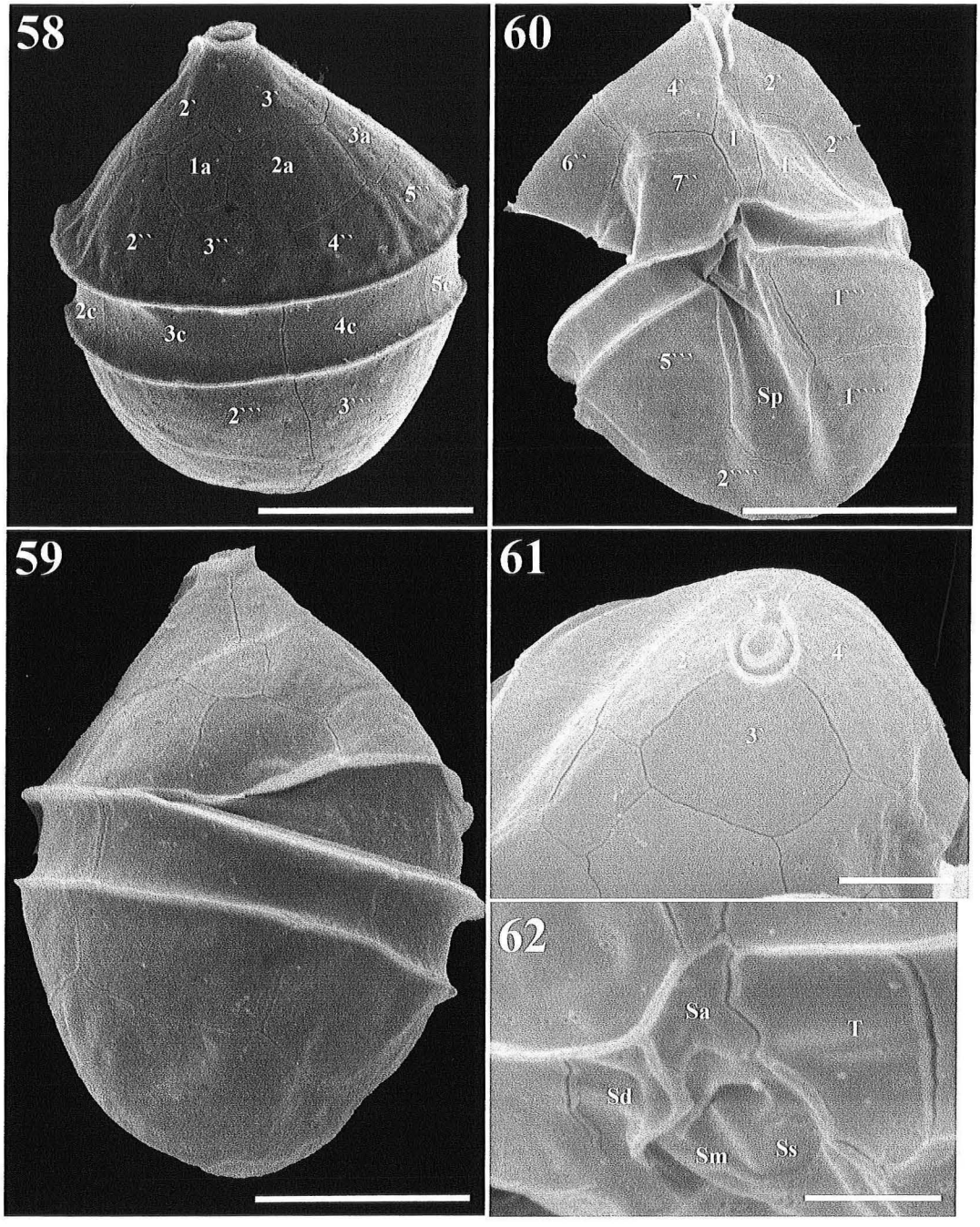
Figs 54-57. LM. Resting cyst of *Scrippsiella* sp. 2.
Fig. 54. Cultured cyst showing calcareous thin outer wall. Note the wall is partially separated from cyst (arrow) by slightly pressure on cover slip.
Fig. 55. Cyst after loss of the outer wall. Note the globular contents.
Fig. 56. Wild cyst showing accumulation body. Note the reniform cyst shape.
Fig. 57. Cyst isolated from sediment showing same network structure (arrows).
All scale bars=10μm.

3.1.4. Scrippsiella trochoidea (Figs 58-76)

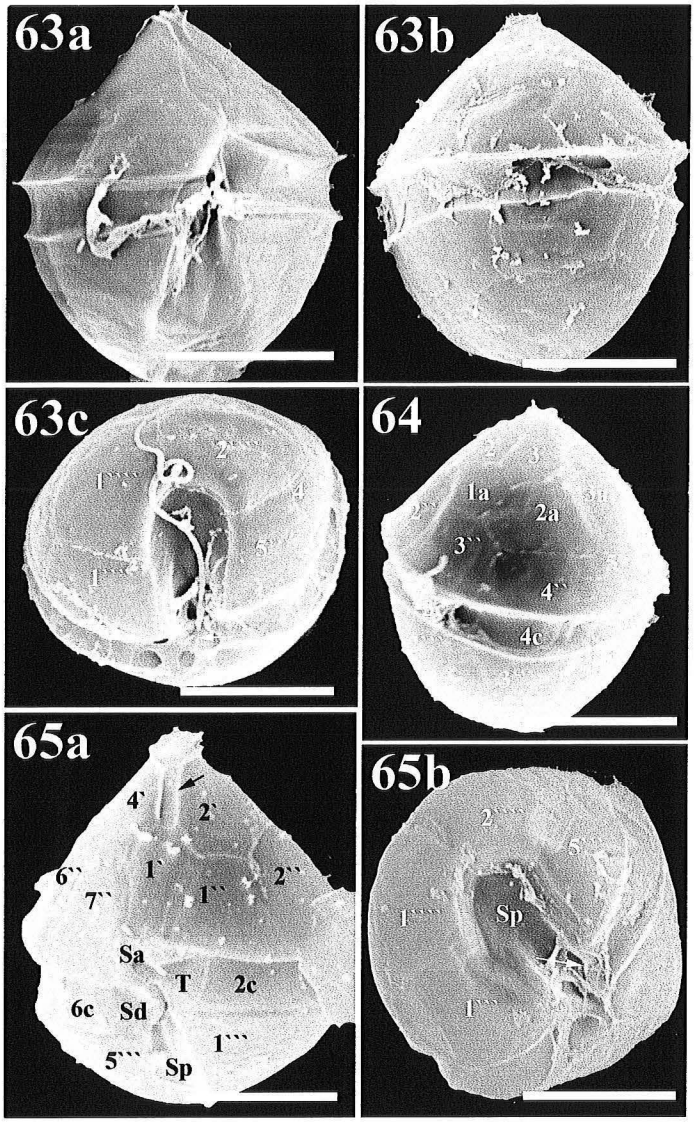
The thecate cells established from five resting cysts (i.e. SCBC18, SCPC36, SCPC39, SCPC51, SCPC73) and all 20 uni-algal cultures established from sediment incubations possessed the same morphology and thecal plate pattern as *Scrippsiella trochoidea*, as described by Lewis (1991). Most strains possessed a conical epitheca with straight sides and a well pronounced apical horn (Figs 58, 63, 65 & 76). Cell sizes range from 18-34μm in length (average=24μm, n=50) and 17-29μm in width (average =22μm, n=50). Numerous simple trichocyst pores are scattered on the thecal plates. The epitheca is slightly larger than the hypotheca.

The plate tabulation is Po, X, 4', 3a, 7'', 5C+1 transitional plate, 5S, 5''', 2'''' (Figs 58-66). The first apical plate is relatively broad and rhomboidal and slightly asymmetrical (Figs 60, 63a & 66). The pore plate is circular and surrounded by a high collar (Figs 58-61, 65 & 66) connected to the first apical plate by a long well-defined canal plate. Five sulcal plates are present. The S_m plate is not completely hidden by the S_d plate (Fig. 62). The sulcus is deeply excavated and broadens antapically (60, 63c, 65). The nucleus is spherical and centrally positioned (Fig71). The globular to ribbon shapes chloroplasts are peripherally placed (Fig71).

Resting cysts are spherical to oval in shape, and range from 18-37μm in diameter. The cyst wall is covered by calcareous spines that extend from a polygonal basal plate and are triangular in cross-section. The spines range from 1.6-4μm in length. All cysts successfully germinated did not possess spines (Fig 67, 68 & 70). The archeopyle is an irregular split about 1/4 of the cyst diameter, with a cap-shaped operculum remaining attached (Figs 74 & 76). Cysts are dark grey-brown in colour. A red accumulation body is visible in intact cysts (Fig. 72).



Figs 58-62. SEM. *Scrippsiella trochoidea* (strain SCBC18).
Fig. 58. Dorsal view showing the plate pattern.
Fig. 59. Dorso-lateral view of the cell.
Fig. 60. Ventral view showing plate pattern.
Fig. 61. Detail of apical region.
Fig. 62. Detail of sulcal plates.
All scale bars=10μm, except Fig. 61 and Fig. 62 scale bars=5μm and 2μm respectively.



Figs 63-65. SEM. *Scrippsiella trochoidea* thecate cells.

Fig. 63a. Ventral view of strain SCPC36.

Fig. 63b. Dorsal view of strain SCPC36.

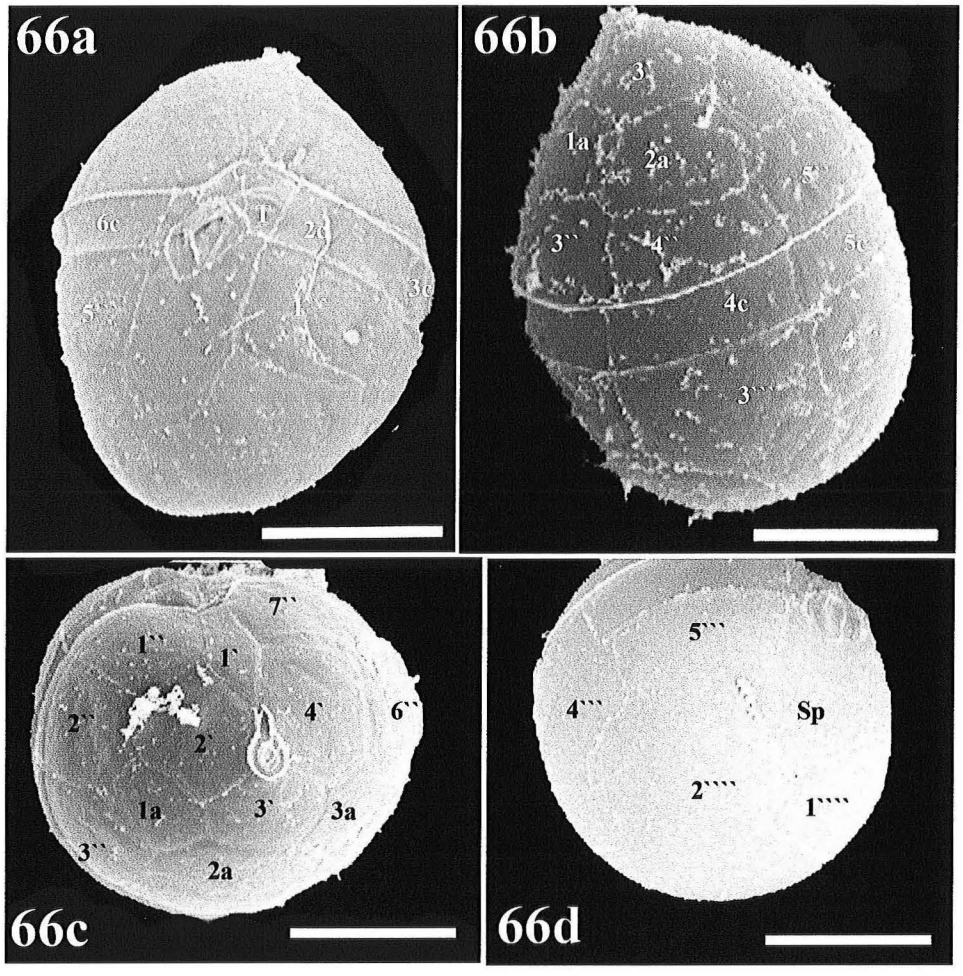
Fig. 63c. Antapical view of strain SCPC36.

Fig. 64. Dorsal view of strain SCPC39.

Fig. 65a. Ventral view of strain SCPC51. Note pronounced canal plate (arrow).

Fig. 65b. Antapical view of the same strain. Arrow showing S_m plate.

All scale bars=10μm.



Figs 66a-d. SEM. Thecate cell of *Scrippsiella trochoidea* strain SCPC73. Stripping and cell swelling technique was used.

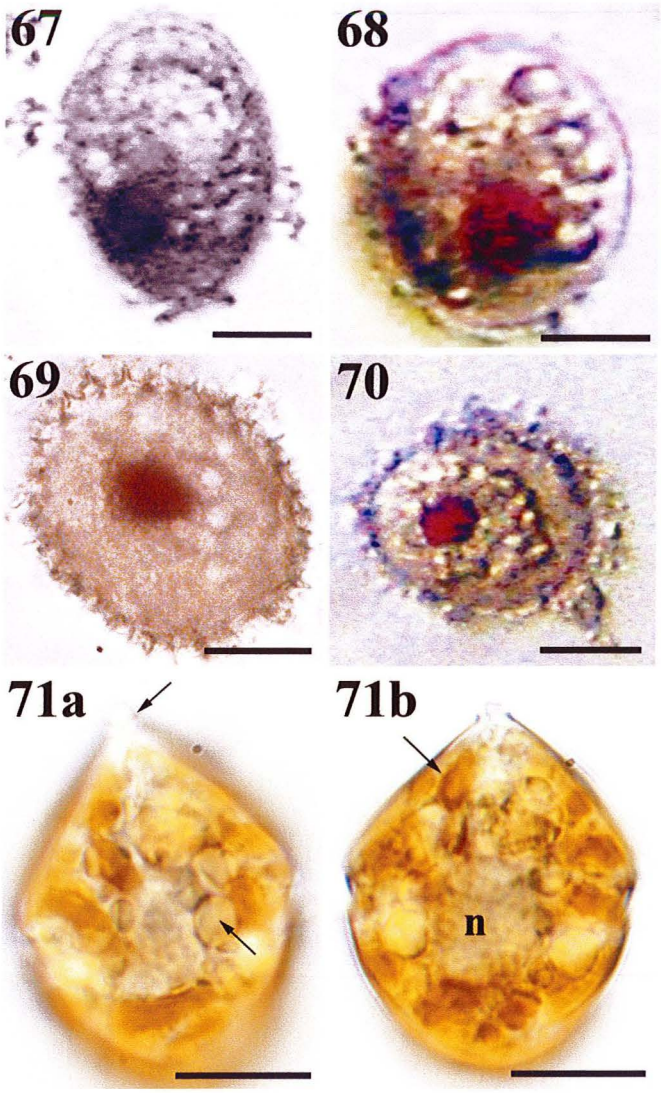
Fig. 66a. Ventral view of the cell.

Fig. 66b. Dorsal view of the cell.

Fig. 66c. Apical view of the cell.

Fig. 66d. Antapical view of the cell.

All scale bars=10μm.



Figs 67-71. LM. Resting cysts of *Scrippsiella trochoidea*.

Fig. 67. Wild resting cyst germinated to establish strain SCBC18.

Fig. 68. Wild resting cyst germinated to establish strain SCPC36. Note lack of spines.

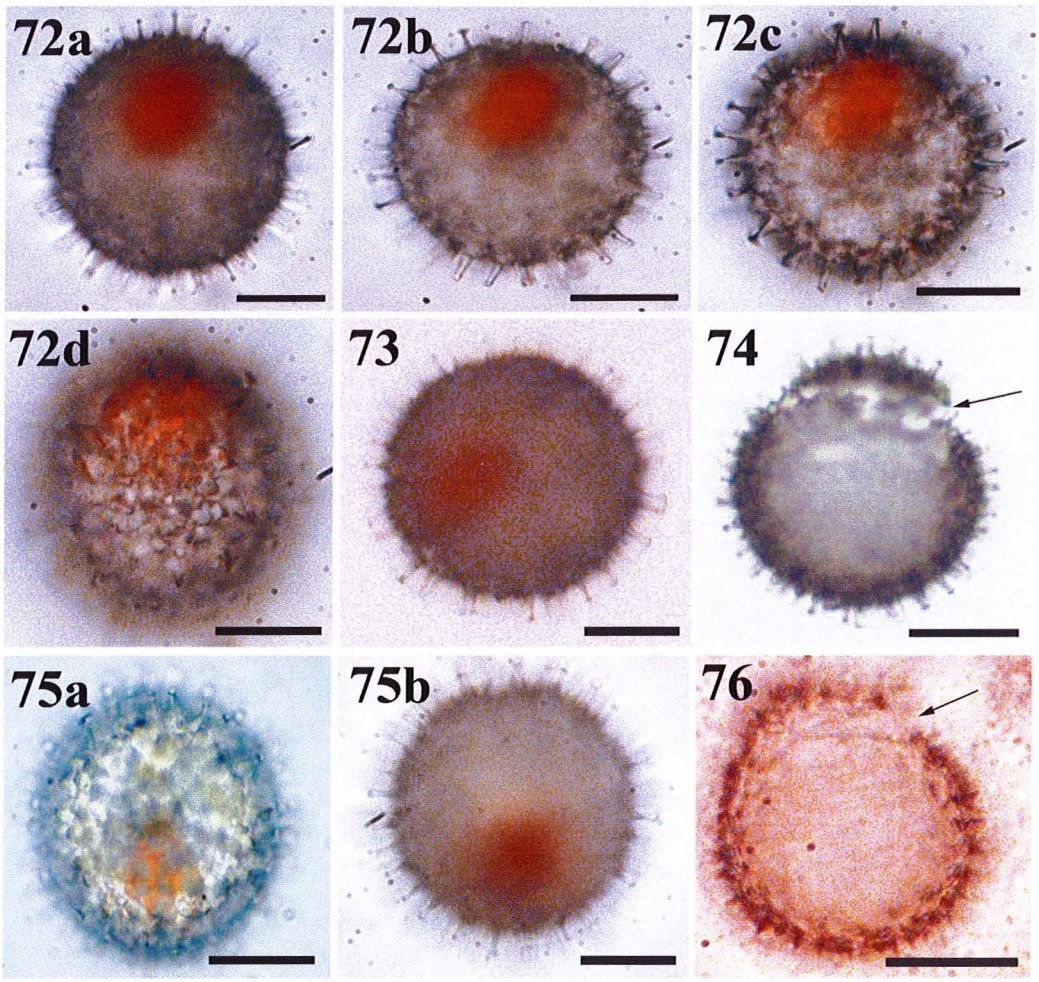
Fig. 69. Resting cyst of *Scrippsiella trochoidea* isolated from sediment.

Fig. 70. Wild resting cyst germinated to establish strain SCPC73.

Fig. 71a. Vegetative cell of strain SCPC51. Note pronounced apical horn (top arrow) and globular chloroplasts (bottom arrow).

Fig. 71b. Dorsal view of cell showing nucleus (n) and chloroplasts (arrow).

All scale bars=10μm.



Figs 72-76. LM. Resting cysts of *Scrippsiella trochoidea* produced in cultures.

Figs 72a-d. Resting cyst with calcareous spines strain SCBC18.

Fig 72a. Spines in deep focus.

Fig 72 b-c. Note the spines in different focus.

Fig. 72d. Spines in surface focus.

Fig.73. Resting cyst of strain SCPC36.

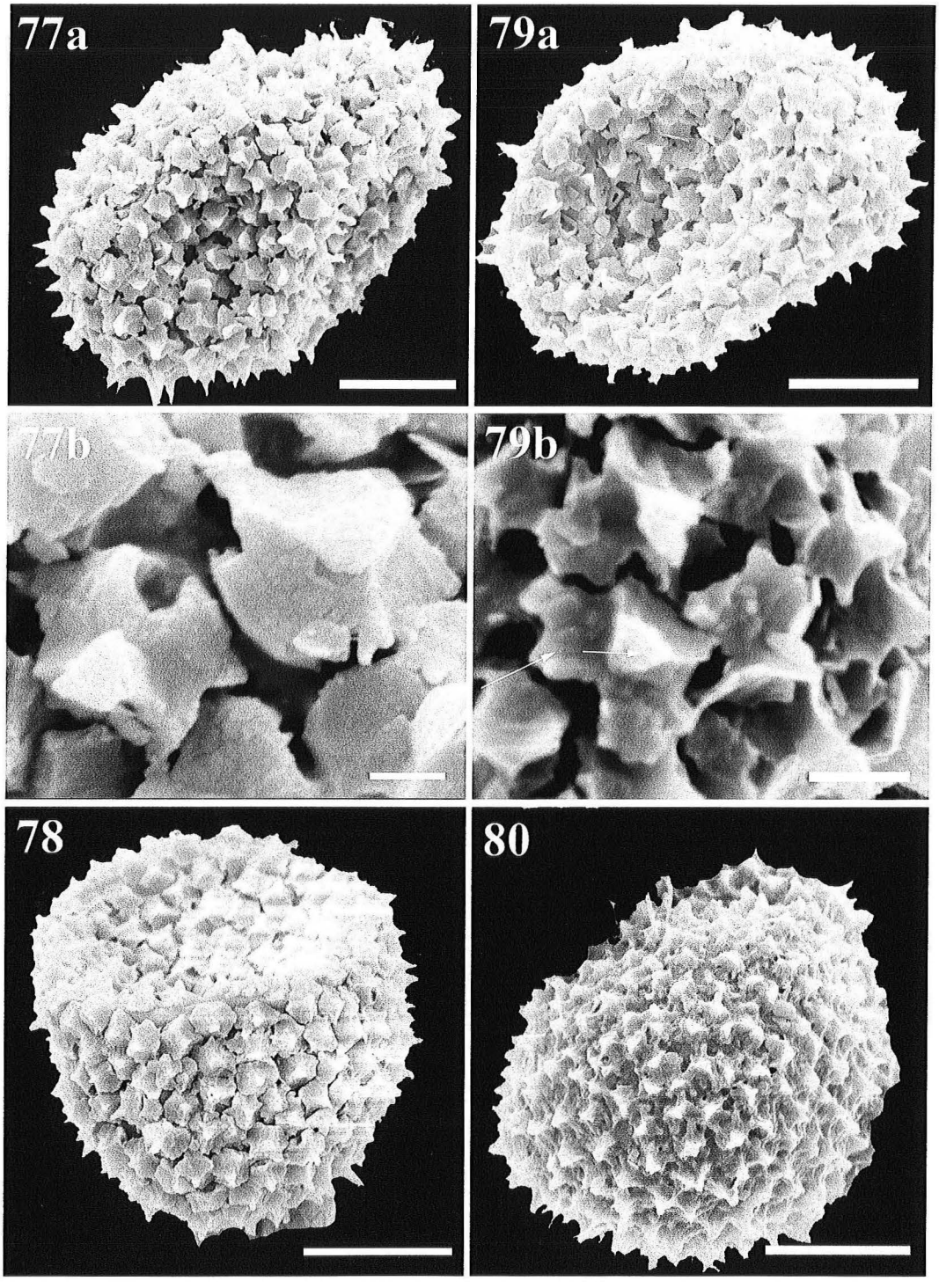
Fig. 74. Resting cyst of strain SCPC39. Note archeopyle (arrow).

Fig.75a. Resting cyst of strain SCPC73, surface focus.

Fig. 75b. Same cyst in deep focus showing spines.

Fig. 76. Resting cyst of strain SCPC51. Note archeopyle (arrow).

All scale bars=10μm.



Figs 77-80. SEM. Resting cyst of *Scrippsiella trochoidea* isolated from sediment.
Fig. 77a. Calcareous cyst with triangular spines.
Fig. 77b. Detail of spines showing irregular base.
Fig. 78. Calcareous resting cyst.
Fig. 79a. Calcareous oval cyst.
Fig. 79b. Note base of spine and calcareous tip (arrows).
Figs. 80. Calcareous cyst showing three corner spines.
All scale bars=10μm, except Fig. 77b and Fig. 79b=5μm.

3.2. Phylogenetic Analyses

Based on sequencing of the rDNA-ITS region, 19 strains established from single cells from sediment incubation were identical to the five *Scrippsiella trochoidea* germinated from single cysts, therefore, these sequences were not included in phylogenetic analyses. Nine of the ten remaining sequences *Scrippsiella* from this study, together with 44 other *Scrippsiella*-like species (including extant species and fossil based-taxa) available from GenBank were phylogenetically analysed based on rDNA-ITS sequences.

The final rDNA-ITS sequence alignment contained 53 taxa and 757 characters (including gaps introduced into the alignment). Significant negative skewness was observed in the tree length distribution of random NJ trees with 56 taxa ($g1=-0.511$, $P<0.01$) and maximum parsimony with 27 taxa ($g1=-0.784$, $P< 0.01$), suggesting that the datasets were phylogenetically informative and more structured than random data (Hillis & Huelsenbeck 1992). Both NJ and MP analyses produced trees with similar branching topology. The NJ analysis (Fig. 81) resolved two major clades: the first clade comprised *Pentapharsodinium dalei* and *Ensiculifera* species; the second clade included all *Scrippsiella*-like species (i.e. this clade is referred to as SCR clade hereafter). Both clades were highly supported by bootstrap analyses (100% and 92% respectively). *Scrippsiella hangoei* occupied a basal position in both analyses, clustering outside the *Scrippsiella*-like species (SCR clade) in both analyses. The SCR clade included *Scrippsiella, Calciodinellum, Calcigonellum* and *Pernambugia.*

Within the SCR clade, three major sub-clades are evident; these are referred to as SCR.A, SCR.B, and SCR.C hereafter (Fig. 81). In the distance tree, the first branching group in the SCR clade is the SCR.A clade that includes *Scrippsiella precaria, S. ramonii* and two strains of *Scrippsiella irregularis* (from the present study). This clade is 100% supported by bootstrap analysis.

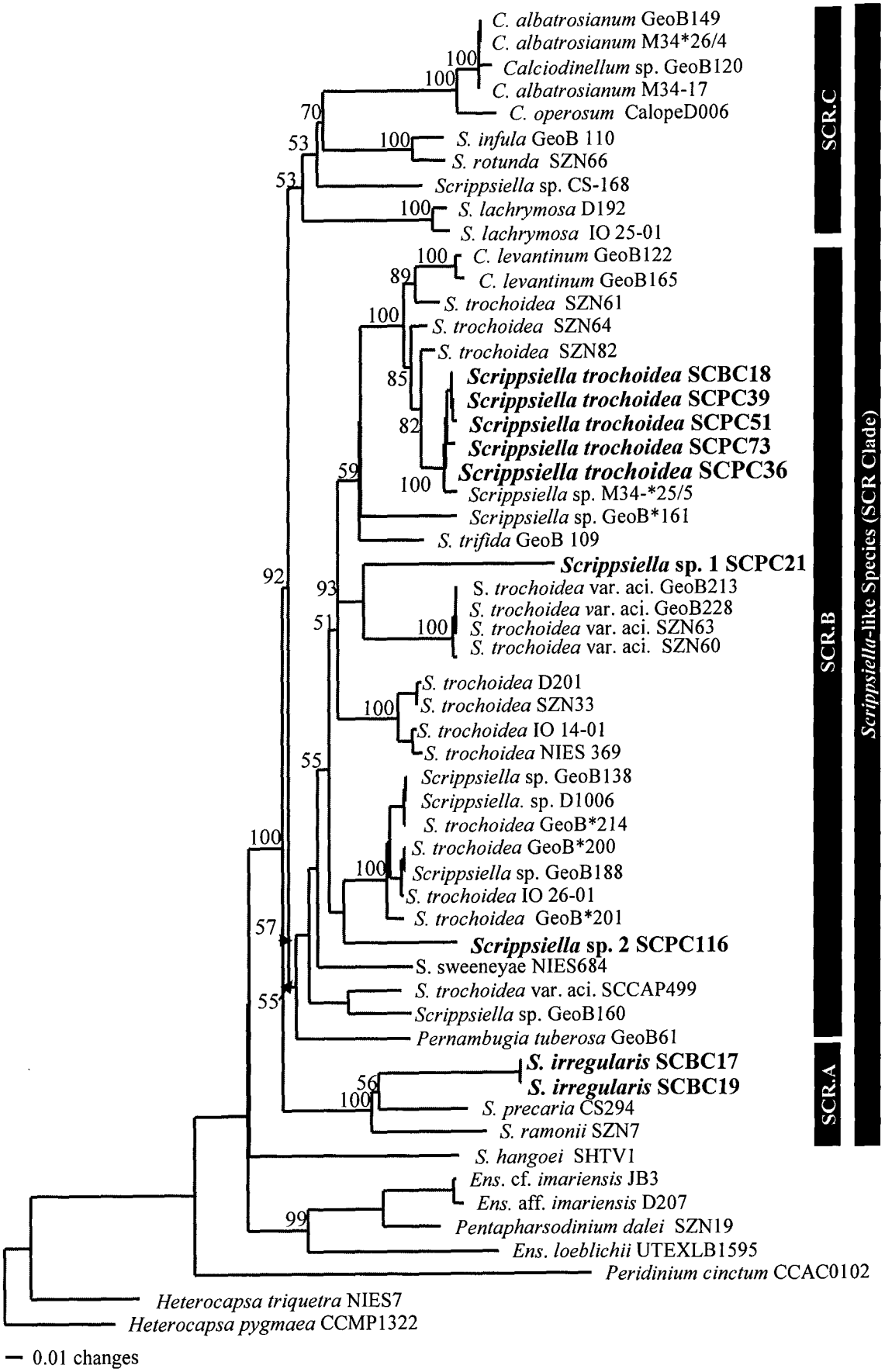


Fig. 81. Neighbour-joining (NJ) phylogeny of 56 taxa from the Calciodinellaceae inferred from sequences of the 5.8s rRNA gene and ITS1 and ITS2 regions. The SCR clade comprises all *Scrippsiella*-like species that include three major subclades (SCR.A, SCR.B, SCR.C). Species from the present study are in bold.

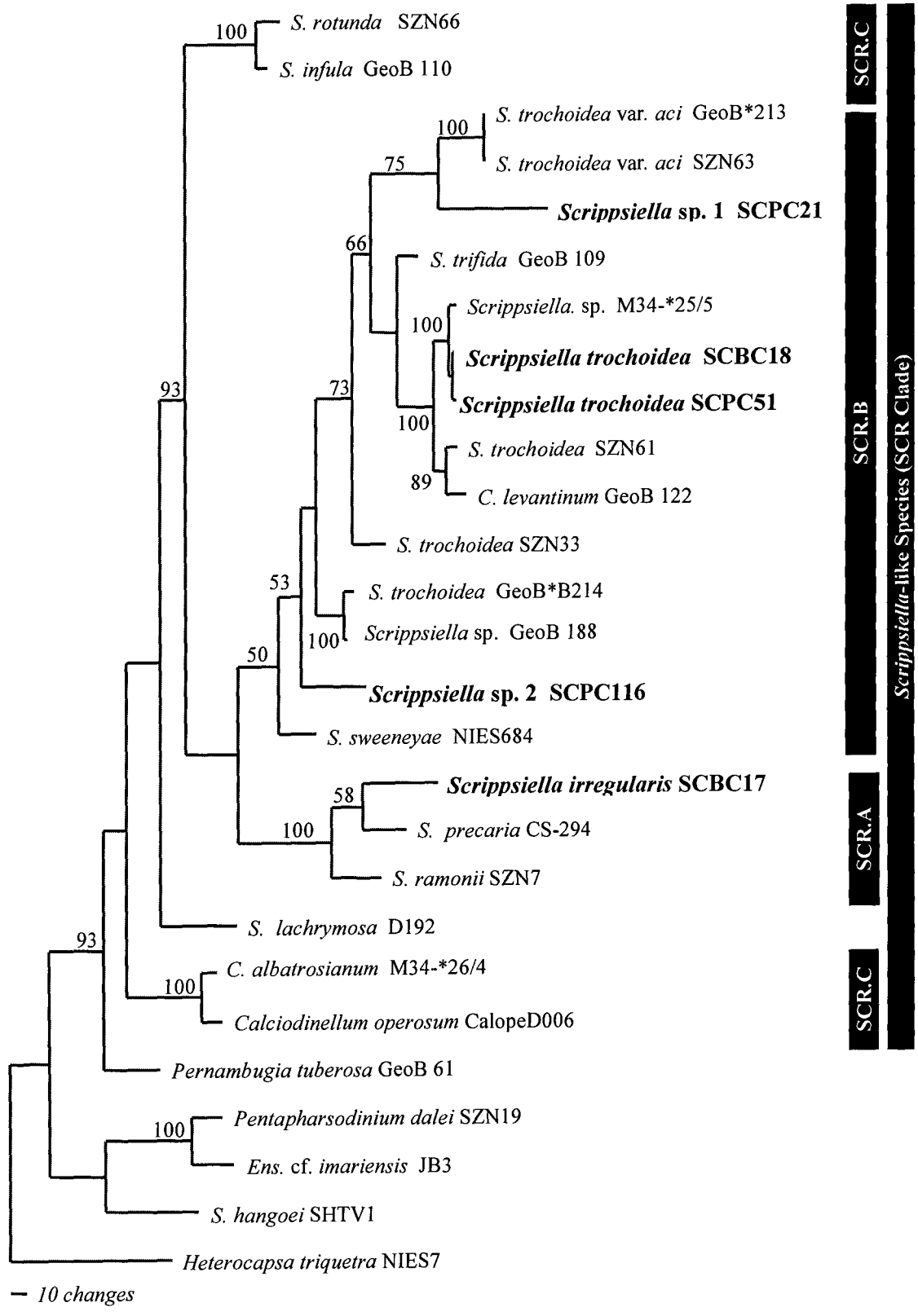


Fig. 82. Most parsimonious tree from a maximum parsimony analysis of 26 taxa from the Calciodinellaceae inferred from sequences of the 5.8S rRNA gene and ITS1 and ITS2 regions. Values at branch points indicate bootstrap support (>50%). *Heterocapsa triquetra* was used as the outgroup taxon. Species from the present study are in bold.

Subclade SCR.B includes all *Scrippsiella trochoidea* complex species, *Calciodinellum levantinum* and *Pernambugia tuberosa.* The SCR.B clade is only weakly supported (55%). The phylogenetic position of *S. sweeneyae* within the clade is uncertain. *Pernambugia tuberosa* clustered at the base of the *Scrippsiella trochoidea* complex clade, but with weak support (57%).

Subclade SRC.C is the second branching group and includes all *Calciodinellum albatrosianum, C. operosum, Calciodinellum* sp., *Scrippsiella infula* and *Scrippsiella rotunda* form a closely related sister group to *Calciodinellum* species with 70% bootstrap support. *Scrippsiella lachrymosa* clusters with *S. infula* and *S. rotunda* but with very weak bootstrap (53%) support.

All *Scrippsiella* species from Iranian waters cluster within the SCR clade into either subclade SCR.A or SCR.B. *Scrippsiella irregularis* strains clusters with *S. precaria* and *S. ramonii* with high bootstrap support (100%), but is clearly distinct. The sequence divergence of *S. irregularis*,, from *S. precaria* and *S. ramonii* is 15.5% and 17% respectively. *Scrippsiella* sp. 1 (Strain SCPC21) clusters with *S. trochoidea* var. *aciculifera* (in SCR.B subclade) with 93% bootstrap support, however, this strain is relatively divergent (18.4%) from its closest relative (strain GeoB*213). *Scrippsiella* sp. 2 is genetically distinct with it nearest relatives in the analyses being *S. trochoidea* strain GeoB*201 (10% seq. divergence) and *S. sweeneyae* (13% seq. divergence). While *Scrippsiella* sp. 2 SCPC116 clusters with the *S. trochoidea* complex species, the bootstrap support is low (57%) and the phylogenetic position of this species is uncertain.

The five remaining strains from this study (SCBC18, SCPC36, SCPC39, SCPC51, and SCPC73) are virtually identical and considered as *Scrippsiella trochoidea* in this study. These strains are cluster with *Scrippsiella* sp. (AY499531) *Scrippsiella trochoidea* species and *Calciodinellum levantinum* species with 100% bootstrap support. The closest relative is *Scrippsiella* sp. strain M34-*25/5 (1.2-1.4% sequence divergence).

The maximum parsimony analysis with 27 taxa (Fig. 82) shows similar branching to the NJ analysis and most clusters in the NJ are also evident in the MP tree. The *Ensiculifera*-like species and *Scrippsiella*-like species (SRC), which are highly supported by bootstrap value of 100% and 95%. In this analysis, *Scrippsiella hangoei* clustered with *Ensiculifera* but with low suppport and its phylogenetic position is uncertain. The *Scrippsiella*-like clade was separated into the three main subclades of SCR.A, SCR.B and SCR.C. The phylogenetic positions of the 9 Iranian strains were similar to that found from the NJ analysis.

4. Discussion

4.1. Phylogenetic Relationship and Morphological Comparisons among the Calciodinellaceae

The phylogenetic relationship of many species of *Scrippsiella* and Scrippsiella-like species is poorly resolved. The genus *Scrippsiella* is considered a subfamily of Peridiniaceae by Fensome *et al.* (1993). Phylogenetic analyses of dinoflagellate genera by Saunders *et al.* (1997) and Daugbjerg *et al.* (2000) show that based on 18S rDNA, genera belonging to the Gymnodiniales, Prorocentrales and Peridiniales are poorly resolved and clustered together. This group was termed the "GPP complex" (*sensu* Sanders et al. 1997). These studies included few calcareous dinoflagellates, however, both *Pentapharsodinium tyrrhenicum* and *Scrippsiella trochoidea* were included, but their precise phylogenetic position, and their relation to the *Peridinium* remained unresolved. Subsequently, D'Onofrio *et al.* (1999), demonstrated the close relationship between calcareous cyst producers (*Pentapharsodinium tyrrhenicum*) and the non-calcareous cyst producers *Ensiculifera imariensis* and *Pentapharsodinium dalei*). In addition, D'Onofrio *et al.* (1999), Montresor *et al.* (2003) and Gottschling *et al.* (2005a, 2005b) have shown that the Calciodinellaceae are a monophyletic clade comprising two groups firstly *Pentapharsodinium* and *Ensiculifera*, and another group including all *Scrippsiella*-like species. In the latter clade, all species possessing the typical *Scrippsiella* thecal plate pattern cluster together, including the fossil-based species *Calciodinellum operosum*, *Calcigonellum infula* and the extant *Scrippsiella* species (it is referred to as *Scrippsiella*-like clade

hereafter). Later molecular studies transferred these two species to *Scrippsiella operosa* (Deflandre) Montresor (basionym: *Calciodinellum operosum* Deflandre) and *Scrippsiella infula* (Deflandre) Montresor (basionym: *Calcigonellum infula* Deflandre) (Montresor *et al.* 2003). The phylogenetic relationships within the *Scrippsiella*-like species and the *Scrippsiella trochoidea* complex remains poorly resolved (Montresor *et al.* 2003) and the phylogenetic position of some species (e.g. as *Scrippsiella sweeneyae* (Balech) Loeblich) remains unclear.

The present molecular study based on sequence analyses of the ITS region of rDNA of Calciodinellaceae agrees with previous molecular study of this group (D'Onofrio *et al.* 1999; Montresor *et al.* 2003; Gottschling *et al.* 2005a, 2005b). The primitive lineage of Calciodinellaceae is *Pentapharsodinium* and *Ensiculifera* which is sister group to the *Scrippsiella*-like species. The present study also shows that all species possessing *Scrippsiella* plate patterns form a monophyletic lineage, confirming the taxonomic value of the number/arrangement of thecal plate at the supra-specific level within this group (Montresor *et al.* 2003). The taxonomy of vegetative cells of these three genera is based on the number of cingular plates and the shape of the first cingular plate (Kobayashi & Matsouka 1995; Steidinger & Tangen 1996). The genus *Scrippsiella* possess six cingular plates (including transitional plate), whereas the other two genera have five cingular plates (including T-plate). The *Ensiculifera* species can also be distinguished from *Pentapharsodinium* genus by the long spine on the first cingular plate (T-plate).

The four groups resolved within the *Scrippsiella* clade, are mostly supported by morphology of the vegetative cells. The most primitive branch in the *Scrippsiella* lineage is the SCR.A group that comprises the *S. precaria*-like species (*S. precaria* Montresor & Zingone, *S. ramonii* Montresor and *S. irregularis* Attaran & Bolch). This monophyletic group is genetically and morphologically distinct from the remaining *Scrippsiella* group. The similarity in the morphology of vegetative cells within these species is highly supported by the phylogenetic analyses. All members in the SCR.A clade have a wider first apical plate than other *Scrippsiella* species. They also possess an asymmetrical arrangement of intercalary plates that is distinct from the symmetrical arrangement of other *Scrippsiella* species (Montresor & Zingone 1988; Montresor 1995). The most important distinguishing feature of the

species in SCR.A clade from other *Scrippsiella* species is the position and size of the second intercalary plate (2a), which is smaller in size and is inserted between the posterior part of the 1a and 3a plates (i.e. 1a and 3a connect to each other).

Within *Scrippsiella*, *S. hangoei* (Schiller) and three benthic *Scrippsiella* species: *S. subsalsa* (Ostenfels) Steidinger & Balech, *S. arenicola* Horiguchi & Pienaar and *S. caponii* (Horiguchi) Pienaar, possess asymmetrical intercalary plates (Larsen *et al.* 1995; Faust 1996). However, *S. hangoei* is a cold water species that produces organic cysts possessing spines with star-shaped tips (Larsen *et al.* 1995). The morphological distinctiveness of this species is supported by the data presented here. This species is not monophyletic with other *Scrippsiella* and has unclear phylogenetic position. Other species in the genus possess a hexagonal 2a plate inserted between 1a and 3a (i.e. 1a and 3a do not connect with each other).

The SRC.B subclade, which is a paraphyletic groups with weak bootstrap support (51%), includes *S. trochoidea* complex, and the phylogenetic relationship among these groups is still unresolved (Montresor *et al.* 2003; Gottschling *et al.* 2005a, 2005b). However, species that are more or less similar in cell morphology or resting cysts morphology are grouped together in phylogenetic analyses. *Pernambugia tuberosa* is clearly distinct from the remaining *Scrippsiella* because of its archeopyle shape (i.e. complete epicyst) (Karwath *et al.* 2000; Gottschling *et al.* 2005a). While the cell morphology of *S. lachrymosa* Lewis is mostly similar to the other members in the subclade SCR.B, the cysts morphology is distinct due to the elongate shape cysts irregular crystalline processes (Lewis 1991), The presence of *Calciodinellum levantinum* Meier, Janofske & Willems, within *Scrippsiella trochoidea* complex group is also not surprising as its thecal tabulation is essentially identical to other *Scrippsiella* (Meier *et al.* 2002).

Scrippsiella rotunda Lewis and *Scrippsiella infula* form a monophyletic group with *Scrippsiella operosa* and *C. albatrosianum* (Kamptner) Janofske & Karwath, and all cluster in subclade SCR.C with weak bootstrap support. Motile cells of these species possess very similar morphology and their plate patterns exactly match the plate patterns of *Scrippsiella* (Montresor *et al.* 2003). All three have poorly developed apical horns, smooth thecal plates with a scattered pore, and a similar nucleus

position (Lewis 1991; Montresor *et al.* 1997). All species in this subclade produce smooth cysts without spines. In describing *S. operosa* (=*Calciodinellum operosum*), Montresor *et al.* (1997) mentioned that *S. operosa* and *S. rotunda* show similarity in the morphology of motile cells and in producing a yellow water-soluble compound in culture. They can therefore only be distinguished by the morphology of their cysts. *S. operosa* produce cysts with pronounced paratabulation. Although Gottschling *et al.* (2005a) found variabilities in producing calcareous cysts in culture of *S. operosa* and *C. albatrosianum*, both species produce cysts with and without clear paratabulation. However, they stated that most strains of *C. albatrosianum* are without clear paratabulation, whereas most strains of *S. operosa* produce cysts with clear paratabulation.

In summary, the molecular analyses presented support the morphological separation of *Scrippsiella*-like species. The ITS-rDNA sequence of all *Scrippsiella*-like species resolved three clusters. One group consists of species that have asymmetrical epithecal plate patterns in their motile stage and spherical-ovoid cysts with numerous and slightly capitate spines (SCR.A, *S. precaria*-like). The second group (SCR.C clade) have smooth-walled resting cysts without spines. The third group (SCR.B clade) is phylogenetically and morphologically highly variable and contains several lineages that do not display common morphological features in either motile cells or resting cysts in each lineage.

4.2. Phylogenetic Relationship and Morphological Comparison of the Species from the Present Study

The four identified morphospecies in the present study fall clearly within different sub-clades within the *Scrippsiella*-like species supporting the separation of the observed morphotypes into four distinct species. To clarify the relationship of each morpho/genotype with existing *Scrippsiella* species, they are each compared with morphologically genetically most similar species in the genus.

Scrippsiella irregularis sp. nov. (strain SCBC17 and SCBC19) is clustered with *S. precaria* and *S. ramonii*. This subclade is highly supported by bootstrap value

(100%). However genetic distance between *S. irregularis* and *S. precaria* and *S. ramonii* appears to be high, with a divergence of 15.5 % and 17% respectively. Surprisingly, S. *precaria* and *S. ramonii* (13.1%) are more similar to each other than to *S. irregularis* and the molecular data strongly support morphological dissimilarity between the motile cells of *S. irregularis* and two other species.

The main features separating *S. irregularis* from its closest relative (*S. precaria)* are summarised in Table 4 and Fig. 83. The thecal plate patterns and overall morphology of *Scrippsiella irregularis* is markedly similar to *Scrippsiella precaria* described by Montresor & Zingone (1988) and *Scrippsiella ramonii* described by Montresor (1995). All three species: *S. precaria, S. ramonii* and *S. irregularis* possess cinctioid tabulation in the dorsal epical plates as discussed above. *S. ramonii* is clearly distinguished from the other two species by an antapical horn on the 2"" plate.

The cell outline, plate patterns and cytological features are important characters for identification of the species (Montresor *et al.* 2003). The vegetative cell of *S. irregularis* is more dome-shaped and resembles *S. ramonii* more than *S. precaria* which is more elongated. The size of the 2a plate is larger than *S. precaria* but smaller than *S. ramonii*, while the subrectangular 2a plate (with 2 curved sides) is similar to that of *S. ramonii* rather than the angular rhomboidal plate of *S. precaria.* The cingulum of *S. irregularis* is narrower that *S. precaria* and the cingular displacement is less than *S. precaria.* The nucleus of *S. precaria* is located in the anterior part of the cell, reaching its equatorial plane (Montresor & Zingone 1988), whereas in *S. irregularis* it is located in the posterior part of the cell in hypotheca (Fig. 12). The transitional plate extends to the first apical plate in *S. precaria* (Fig. 1a, Montresor & Zingone 1988), but does not extend in *S. irregularis.* Cells of *S. irregularis* are generally larger in size than *S. precaria*, although the size ranges of both species overlap considerably (Table 4).

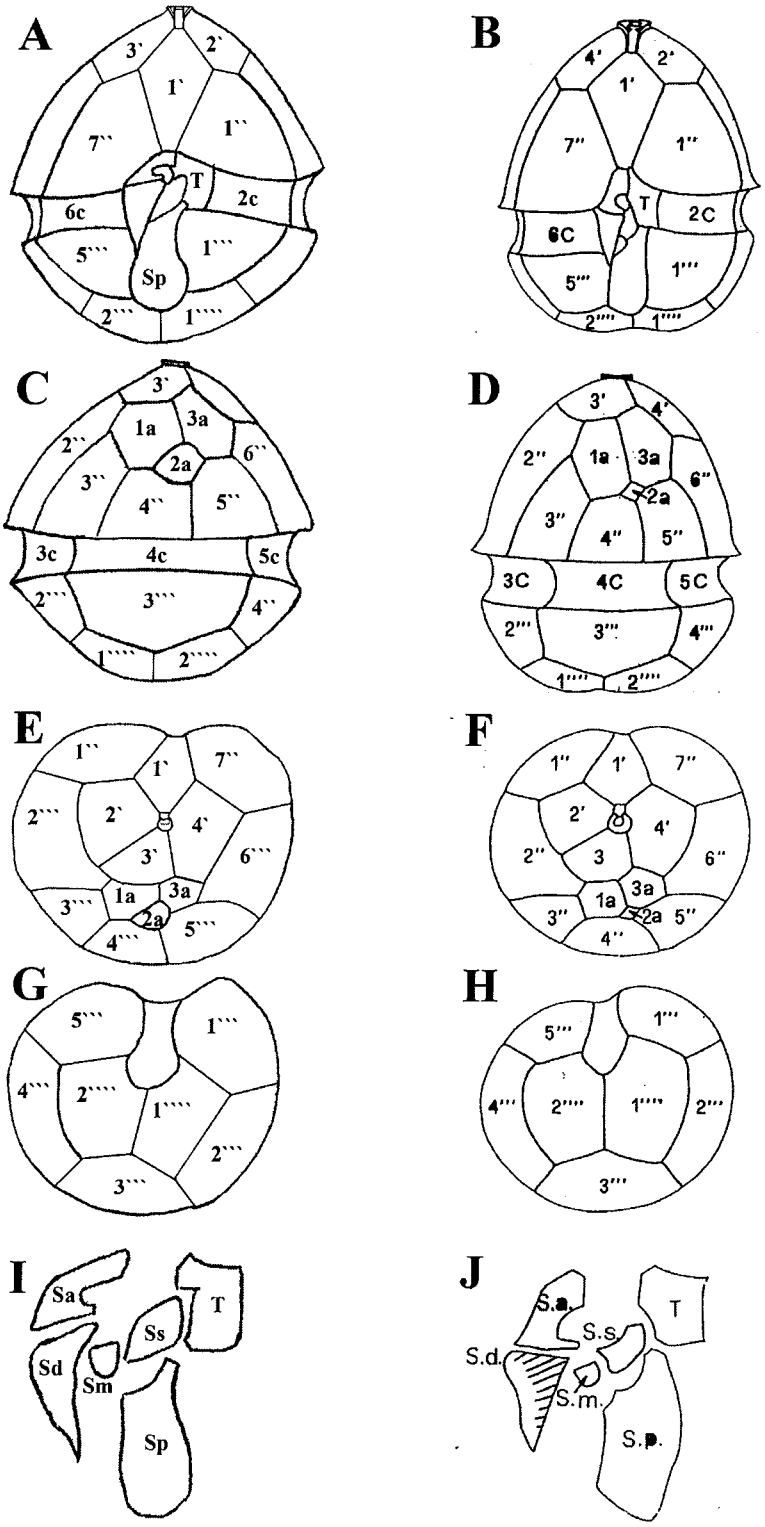
The cysts of both *S. precaria* and *S. irregularis* are spiny and calcareous, however *S. irregularis* cysts are slightly larger and a grey colour compared to the light brown colour of *S. precaria* cysts (Table 4). Cultured cysts of *S. irregularis* possess an outer membrane surrounding the capitate spines, which has not been reported for *S. precaria,* although this membrane probably degrades in natural sediment. While the

cysts of *S. ramonii* are similar, they can be distinguished *S. precaria* and *S. irregularis* by the dark brown colour and much longer processes (Montresor 1995).

Table 4: Morphological comparisons of motile cells and cysts of *Scrippsiella irregularis* with *S. precaria and S. ramonii*

Motile cell	*S. ramonii*[c] Montresor	*S. precaria*[b] Montresor & Zingone	*S. irregularis*[a] *Attaran-Fariman & Bolch*
Motile Cell			
Length µm	22-34	15-25 (mean=19.2)	17-29 (mean=22)
Width µm	19-27	13.8-20 (mean=16)	13-23 (mean=18)
Cingulum width µm	?	mean=4.1	2.6-3.6 (mean=2.9)
Cingulum displacement	?	2/3 of its width	1/2-1 of its width
Apex	Rounded	Rounded	Rounded
Antapex	Small horn	Flattened	Rounded
Epitheca/hypotheca mean ratio	? >1.0	1.6	1.4
2a plate; Size	Large	Small	Medium
Shape	Rhombic	Rhomboidal	Rounded sub-rectangular
Nucleus; Shape	Spherical	Spherical	Spherical
Position	Central	Anterior	Posterior
Colour	Yellow-green	Olive-green	Brown-green
Cyst stage			
Size (µm)	31-36 long 25-26 wide	17.5-25(mean=20.5) 15-23 (mean=18.6)	20-26 in diameter (mean=24)
Shape	ovoid	Spherical to oval	Spherical to sub-spherical
Colour	Dark brown	Light brown	Light brown- grey
Ornamentation	Capitate	Generally pointed, sometimes capitate	Pointed or capitate spines
Process length	9-11.5	3.1-6.9 (mean=4.7)	3.6-4.5 (mean=4)

([a]) Present study ([b]) Montresor & Zingone (1988) ([c]) , Montresor (1995)



Scrippsiella irregularis ***Scrippsiella precaria***

Fig. 83. Schematic drawing of *Scrippsiella irregularis* theca cell, compared with *Scrippsiella precaria*. A, B. ventral view; C, D. dorsal view; E, F. apical view; G, H. antapical view; and I, J. sulcal plates (Drawing of *S. precaria* is adapted from Montresor & Zingone 1988)

Scrippsiella sp. 1 (SCPC21) is genetically allied to *Scrippsiella trochoidea* var. *aciculifera* Montresor in the phylogenetic analyses and nested within the *Scrippsiella trochoidea* complex. The genetic distance of strain SCPC21 from S. *trochoidea* var. *aciculifera* is high, with a divergence of 18.4-18.8% suggesting that this morphotype is a distinct species.

Both strains SCPC21 and SCPC23 species differ from *S. trochoidea* var. *aciculifera* by the absence of a spine on the anterior sulcal plate. A diagnostic feature of *S. trochoidea* var. *aciculifera* is the presence of a 4-6μm spine on the S_a plate, usually visible only hypothecae that have been separated from the epitheca (D'Onofrio *et al.* 1999; Montresor *et al.* 2003). This raises two questions: 1) is a spine on S_a plate a stable character separating *S. trochoidea* var. *aciculifera* from other *Scrippsiella trochoidea*? or 2) Is SCPC21 a new species allied to *S. trochoidea* var. *aciculifera* ?

The nucleus is positioned centrally in this species, similar to other *S. trochoidea* and S. *trochoidea* var. *aciculifera* .However, there are differences between SCPC21/23 strains and both S. *trochoidea* and S. *trochoidea* var. *aciculifera.* A small but consistent v-shaped split is evident on the left side of the apical horn ridge, a feature not reported in either *S. trochoidea* or *S. trochoidea* var. *aciculifera* (Lewis 1991; D'Onofrio *et al.* 1999; Janofske 2000; and *Montresor et al.* 2003). Strains SCPC21 and 23 also produce dark greenish-brown soluble compounds in the culture medium. A yellow-water soluble compound has been reported in *S. tinctoria* Indelicato & Loeblich, *S. rotunda* and *S. operosum* (Indelicato & Loeblich 1986; Lewis 1991; Montresor *et al.* 1997), however, there is some dispute over the taxonomic validity of this character. It is considered as an important feature by Indelicato & Loeblich (1985), but not an important taxonomic feature by Lewis (1991).

Among the 30 strains of *Scrippsiella* spp. established in this work, only three produced dark greenish-brown soluble compounds. This colour was also observed even with very low cells concentration). Strain SCPC13, established by isolation of individual cell (SCPI3) from mixed incubated sediments, also produced similar coloured dissolved compounds and was morphologically and genetically similar to strains SCPC21, SCPC23. This would suggest that this character can be a useful

feature for distinguishing individual *Scrippsiella* species, but that it is not a reliable marker for particular *Scrippsiella* lineages distinguished in the phylogenetic analyses.

The cysts of strains SCPC21 and SCPC23 either have irregular spines or more commonly are found without spines, but covered with irregular-shaped crystals. Cysts of *S. trochoidea* var. *aciculifera* and *S. trochoidea*, in contrast, both have calcareous triangular spines of various lengths with an irregular basal plate of similar shape (D'Onofrio *et al.* 1999; Janofske 2000; Montresor *et al.* 2003). However, under laboratory culture conditions, *S. trochoidea* and *S. trochoidea* var. *aciculifera* are known to produce cysts with a variety of calcareous ornamentation (Wall *et al.*1970; Lewis 1991; D'Onofrio *et al.*1999; Montresor *et al.* 2003). Cultures in this study also show variation in the morphology of ornamentation, from well-spaced crystals with irregular shapes, to more tightly packed polygonal crystals. In addition the culture of this species was established from a cyst without any morphological features is not clear whether wild cysts have the same morphology as cultured cysts.

Scrippsiella sp. 2 (SCPC 116) has an uncertain phylogenetic position in the *Scrippsiella trochoidea* complex (SCR.B subclade), clustering near the base of the *S. trochoidea* complex, near *S. sweeneyae.* There is much confusion in the taxonomy of the *S. sweeneyae*. It was first described by Balech (1959) from a motile cell from the Pacific Ocean. Subsequently, the resting cyst was described by Wall and Dale (1968) along with the motile stage from the Arabian Sea. The cysts of *S. sweeneyae* are similar to those of *C. operosum* (Montresor *et al.* 1997) in both having a cyst without any spines. In contrast, Janofske (2000) considers *S. sweeneyae* as synonymous with *S. trochoidea.* Given the relatively large divergence of strain SCPC116 from closest relatives (11-13%), *S. trochoidea* (AY676158) and *S. sweeneyae* (AY499520), it is appears more likely that this morphotype represents a distinct species.

The vegetative cell of *Scrippsiella* sp. 2 shares many morphological characters attributed to *Scrippsiella trochoidea.* These are size, arrangement of intercalary plates, precingular, antapical cingulum and sulcal plates. The cell outline of the species is different from *S. trochoidea* described by Lewis (1991) and Janofske (2000), but more resembles *Scrippsiella* cf. *trochoidea* strain SZN 76 (D'Onofrio *et*

al. 1999) with a more rounded epitheca and hypotheca, and a more equatorially-placed cingulum.

The most distinctive feature of SCPC116 is the first apical plate. Most *Scrippsiella* species possess a rhomboidal first apical plate, either wide or narrow, and usually asymmetrical and narrowing to where it meets the cingulum margin. The first apical plate of strain SCPC116 differs by having an anterior part that is rhomboidal in shape while the lower part is rectangular and does not narrow significantly as it nears the sulcus. Cells of strain SCPC116 also show variation in the number of post-cingular plates; some cells have six post-cingular plates instead of the usual five. Similar variations in intercalary or precingular plate number have previously been reported by Lewis (1991) and Gottschling *et al.* (2005a).

The main features of the cyst and motile stages are compared in Table 5. The most important and decisive character distinguishing *Scrippsiella* sp. 2 from other species in the genus is the cyst morphology. The cysts are large, spherical to sub-spherical and smooth-walled and lack paratabulation. They are comparable in size only to *Scrippsiella crystallina* Lewis and *S. sweenyae*. Both the latter produce large, calcareous cysts without spines, but the cyst shape and calcareous pattern is quite different. *S. crystallina* cysts are 50-68μm in length, elongated egg-shaped with a small apex, rounded antapex and ornamented with tightly packed calcareous blocks (Lewis 1991). *S. sweenyae* produces large calcareous cysts without spines and an imperfect paratabulation pattern with a granular texture (Wall & Dale 1968). *Scrippsiella lachrymosa* also produces calcareous cysts with irregular brick-shaped crystal patterns (Lewis 1991), but the cysts of this species are oval and much smaller in size. The network calcitic structures of *Scrippsiella* sp. 2 cysts may be comparable to the tightly packed, rod-shaped crystals of *S. rotunda* Lewis cysts that, also have a smooth appearance under the light microscope (Lewis 1991).

Species in the genus *Calciodinellum* such as *Calciodinellum albatrosianum* Kamptner and *Calciodinellum operosum* also produce smooth cysts with reticulate structures, however these species possess clear paratabulation (Montresor *et al.* 1997; Meier & Willems 2003; Vink 2004), however, the cysts of both species are much smaller (24-28μm). The morphology of motile cells of *S. operosa* can also be

distinguished from strain SCPC116, by having a wider first apical plate and the lack of apical horn.

Table 5: Comparing cyst and motile stage of *Scrippsiella* sp. 2 (SCPC116) with other similar cyst-forming species

Feature	[d]*S. operosa*	[c]*S. sweenyae*	[b]*S. rotunda*	*Scrippsiella* sp. 2[a]
Cyst				
Size µm	24-28 with	40-54 (width) 49-58 (length)	23-40	30-65
Shape	Spherical	Spherical	Spherical	Spherical to subspherical
Ornament	Small crystal spongy-reticulate structure/ with or without clear paratabulation	Microgranular texture, imperfect paratabulation	Calcareous closely packed upright needles	Small crystal with 3-6 sides reticulate structure, no paratabulation
Motile cell				
length µm	20-28	32-36	23-40	22-29
wide µm	18-24	28-29	20-34	20-25
Epitheca	Conical-rounded	Conical-convex sides	Rounded	Conical convex sides
1' plate	Wide	?	Wide	Narrow
Apical horn	Lack	?	Flat collar	Pronounced

([a]) present study ([b]) Lewis 1991 ([c]) Wall & Dale 1968 ([d]) Montresor *et al.* 1997.

Scrippsiella trochoidea (strains SCBC18, SCPC36, SCPC39, SCPC51, SCPC73) from present study shows high similarity to the described *S. trochoidea* (Lewis 1991; Janofske 2000; Montresor *et al.* 2003). Both have similar vegetative cells of similar cell size, bipesioid arrangement of intercalary plates, a narrow to medium width first apical plate, smooth plate surface with scattered pores, a conical epitheca and rounded hypotheca and a central position of the nucleus.

The rDNA-ITS sequence data indicate these strains are closely related to *Scrippsiella* sp. (strain M34-*25/5). This monophyletic group is a sister group to *S. trochoidea* (SZN82) and *S. trochoidea* (SZN64). *Scrippsiella* sp strain M34-*25/5 is not well

characterised (Gottschling *et al.* (2005a) and the only figure of the motile cell (fig 33, dorsal view, Gottschling *et al.* 2005a), shows a cell outline similar to the strains in the present study). The cyst of strain M34-*25/5 is ovoid and covered with regular-shaped crystals without any spines (Gottschling *et al.* 2005a). Previous studies on the phylogeny of calcareous cyst-producing dinoflagellate (e.g. D'Onofrio *et al.* 1999; Montresor *et al.* 2003) have also found that species with obviously different cyst morphology may group together in the phylogenetic trees.

The number, shape and arrangement of the sulcal plates are conservative features in species and valuable characters for the taxonomy of the armoured dinoflagellates (Balech 1980). The number of sulcal plates for *Scrippsiella trochoidea* does not agree with Janofske (2000) who described six sulcal plates and an overall plate pattern of Po, X, 4', 3a, 7", 6C, 6S, 5"', 2"". The extra plate appears to be the anterior flagellar pore plate (af) placed between the anterior sulcal plate (S_a), and the T-plate, slightly overlapped by the right sulcal (rs; S_d). In the present study, five sulcal plates were identified: the anterior (S_a), left (S_s), right (S_d), posterior (S_p) and a small median sulcal plate (S_m). The S_m plate corresponds to the posterior flagellar pore of Janofske (2000). However, the left sulcal plate (S_s), in *Scrippsiella trochoidea* (Fig. 62, strain SCBC18) appears to be folded over the transverse flagellar. Five sulcal plates (S_a, S_s, S_d, S_p & S_m) are also reported in other descriptions for *S. trochoidea* (e.g. Lewis 1991, Steidinger & Tangen 1996; Montresor *et al.* 2003).

The resting cysts of *Scrippsiella trochoidea* in the present study correspond with those previously described for *Scrippsiella trochoidea*, possessing capitate calcareous spines with triangular cross-sections, emerging from an irregular base plate (Janofske 2000). The encystment openings (archeopyle) also match those of *S. trochoidea* therefore, molecular data, morphology of cyst and vegetative cell suggest that this species can be considered *Scrippsiella trochoidea.*

5. Conclusion

Four morphologically distinct *Scrippsiella* species are demonstrated in this study: *Scrippsiella irregularis, Scrippsiella* sp. 1, *Scrippsiella* sp. 2 and *S. trochoidea.*

Scrippsiella irregularis is a new species allied to *Scrippsiella precaria* and *S. ramonii*, but is genetically and morphologically distinct. Although *Scrippsiella* sp. 1 closely related to *S. trochoidea* var. *aciculifera*, it lacks the diagnostic feature of this variety, the spine on anterior sulcal plate and is most likely a distinct species. *Scrippsiella* sp. 2 is clearly distinct from other species in the genus due to the large cyst covered with a network of calcitic crystals that superficially resemble cysts of some gymnodinioid species. This is most likely a new species, but its phylogenetic position is unclear and I have insufficient morphological data to describe it as a new species. Based on the morphology of the cells and cysts, and molecular data, *Scrippsiella trochoidea* from southern Iranian waters corresponds with previous descriptions of *Scrippsiella trochoidea*, further extending the known range of this apparently cosmopolitan species. This study also confirms that molecular studies of the rDNA, if combined with morphology of the cyst and cell studies, are a valuable approach to taxonomy of Calciodinellid dinoflagellates.

6. Further Study

The presence of cryptic species in the genus *Scrippsiella* with highly similar motile cell morphology create significant taxonomic problems. Janofske (2000) suggests that the crystallographic orientation (c-axis) of the calcareous crystal can be a valuable character in the taxonomy of the species in the genus. To date, this orientation has been investigated in some species such as: *S. trochoidea, S. regalis* (Janofske 2000), *S. trifida* (Head *et al.* 2006) and *S. operosa* (Montresor 1997), and it would be valuable to extents this approach to other species in the group. In addition, cyst formation of the species identified in this study was very low in laboratory cultures (under nutrient depletion). It would be valuable to examine the environmental factors regulating their encystment or excystment that may be important in predicting the population dynamics of this group along the south coast of Iran investigated

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CHAPTER 5

Phylogenetic and Morphological Analysis of *Chattonella* cf. *subsalsa* strain CHP36 from Southeast Coast of Iran

1. Introduction

Raphidophytes (Raphidophyceae Chadefaud & Silva =class Chloromonadophyceae Panefuss) are small golden-brown flagellates that possess fucoxanthin pigment as a common pigment. Cells are dorsoventrally compressed and possess two subequal heterodynamic flagella that arise from a more or less developed flagella groove. The cells contain numerous ejectosomes (*Heterosigma* Hada), trichocyst and mucocysts (*Chattonella* Biecheler and *Fibrocapsa* Toriumi & Takano) and numerous golden-brown chloroplasts that are peripherally placed (Hallegraeff & Hara 2003).

Taxonomy of the class is based mainly on cell shape, size, and the ultrastucture of the chloroplasts, mucocysts trichocysts and ejectosomes (Marshall *et al.* 2002), however, the existing taxonomy is still controversial and poorly resolved (Kim *et al.* 1999; Marshall *et al.* 2002). A resting stage (or cyst) has been reported for some species, including *Chattonella antiqua* (Hada) Ona, *Chattonella marina* (Subrahmanian) Hara & Chihara (Yamaguchi & Imai 1994), *Heterosigma akashiwo* (Hada) Hara & Chihara (Smayda 1998), *Fibrocapsa japonica* Toriumi & Takano (Yoshimatsu 1987) and *Chattonella subsalsa* Biecheler (Steidinger & Penta 1999). However, resting cysts of *Chattonella globosa* Hara & Chihara are unknown (Hallegraeff & Hara 2003). These cysts may have a role in bloom initiation in coastal areas (Peperzak 2001).

A number of marine species are known to form dense and potentially harmful blooms in coastal areas (Connell & Jacobs 1997; Kim *et al.* 1999), causing serious problems for aquaculture in many countries, including: Hong Kong, Japan, Canada and Australia (Hallegraeff *et al.* 1998; Tomas 1998; Imai *et al.* 2001; Marshall *et al.* 2000; Tiffany *et al.* 2001; Landesberg 2002; Tang *et al.* 2005). Blooms of *Chattonella antiqua, C. marina, C. subsalsa, Fibrocapsa japonica*, and *Heterosigma akashiwo* have often been associated with mortalities of both cultured and wild fish and shellfish (Oda *et al.* 1997; Kim *et al.* 2000;; Peperzak 2001; Band-Schmidt *et al.* 2004; Hard *et al.* 2000; Hiroishi *et al.* 2005). As a consequence, the accurate identification of members of this class is most important.

Identification of raphidophytes species based solely on morphology is problematic due to their fragile nature, and the pleomorphic morphology of some species (Aizdaicher 1993; Tyrrell *et al.* 2001) leading to difficulties distinguishing one species from another, especially those within the *Chattonella marina/subsalsa/antiqua* complex (Marshall *et al.* 2002). The fragile cells of this group are also difficult to identify by transmission electron microscopy after fixation due to a range of fixation artefacts (Throndsen 1993; Marshall *et al.* 2002).

DNA sequencing has been used to examine relationships among marine microalga including Raphidophyceae taxa, at both the population level and species level (e.g. Potter *et al.* 1997; Connell 2000, 2002). There are relatively few molecular studies based on large subunit (LSU) rDNA from heterokont algae (e.g. Ben Ali *et al.* 2002), however, several studies of raphidophytes have successfully used the rDNA-ITS region to examine raphidophyte phylogeny and there is relatively broad coverage of rDNA-ITS sequence available on public databases. These studies indicate that, while there is variation between species, there is little or no rDNA-ITS sequence variation within species, even among isolates from across the globe (Connell 2000; Kooistra *et al.* 2001; Connell 2002). Therefore this region is a potentially useful means to distinguish distinct species of raphidophytes.

In this chapter, motile cells of a novel *Chattonella* morphotype, strain CHPI36, from the south coast of Iran are described by light and scanning electron microscopy, and

phylogenetic analyses carried out based on LSU-rDNA and rDNA-ITS sequences. The Iranian isolation is shown to be similar to, but genetically distinct from *Chattonella subsalsa* and is either a sub-species of *C. subsalsa* or more likely represents new species of *Chattonella* related to *C. subsalsa.*

2. Material and Methods

2.1. Cell Isolation and Culture

During dinoflagellate cyst surveys from southeast Iranian sediment (see Chapter 2), a golden-yellow flagellate cell was isolated from incubated mixed sediment collected from Pasabandar. Individual cells were isolated by micropipette under a Leica stereomicroscope, placed in a 55mm polystyrene petri dish containing 15ml of GSe medium (FSW with 35% salinity was used), sealed with parafilm (TM) and incubated at 26°C ± 0.5°C, under cool white fluorescent light (70-90µmol photon $m^{-1}s^{-1}$) with a 12h light: 12h dark cycle. Successfully cultured cells were subsequently transferred to 100ml Erlenmeyer flasks containing 50ml of GSe and subcultured every 3 weeks. Duplicate flasks were also held at 17°C ± 0.5°C at the same light intensity.

With the aim of promoting encystment in cultures, one ml of culture of *Chattonella* cf. *subsalsa* strain CHPI36 was inoculated in 55mm petri dishes containing 20ml of a nitrate/phosphate-deficient GSe medium. The petri dishes were sealed with parafilm (TM) and incubated under the same conditions as above. Dishes were checked regularly for evidence of resting cyst production (Yoshimatsu 1987; Imai & Itakura 1999).

2.2. Microscopy

2.2.1. Light Microscopy

Cells were photographed with an Olympus BH-2 microscope equipped with Leica DC300F digital imaging system and also a Zeiss Axioplan 2 Plus microscope (Zeiss, Gottingen, Germany) equipped with Zeiss Axiocam HR digital camera using bright field and differential interference contrast illumination.

2.2.2. Scanning Electron Microscopy (SEM)

Ten ml of mid-logarithmic growth-phase cultures of strains of *C.* cf. *subsalsa* was collected in 14ml centrifuge tubes, with the aim of stressing the cells as little as possible. Samples were gently centrifuged (1000×g) for 5 min. The cells were then fixed for 30 min by the addition of an equal volume of 4% osmium tetroxide (OsO_4) dissolved in 28ppt FSW. Fixed cells were rinsed once with 70% FSW, and twice with the deionised water and then concentrated by centrifuge as above. The concentrated cells were adhered to polylysine coated coverslips (Marchant & Thomas 1983) and dehydrated in a graded methanol series (10-100% in eight steps), critical-point dried via liquid CO_2 (Balzers CPD020, Germany), mounted on SEM stubs, sputter coated with gold (Balzers SPC004, Germany), and examined with a JEOL JSM-840 scanning electron microscope.

2.3. DNA Extraction, PCR and DNA Sequencing

DNA was extracted by the phenol:chloroform:isoamyl alcohol gentle-lysis method (Bolch *et al.* 1998) described in detail in Chapter 3. PCR primers ITSA and ITSB (Adachi *et al.* 1994) were used to amplify the internal transcribed spacer 1 (ITS1), 5.8S rRNA gene and, internal transcribed spacer2 (ITS2) (hereafter referred to as rDNA-ITS). For amplification of the partial large subunit (LSU) region, PCR primers D1R-F (Scholin *et al.* 1994) and 1483-R primers (Daugbjerg *et al.* 2000) were used (see Chapter 3 for detail).

Successful PCR products were purified using Montage PCR clean-up columns (Millipore, USA) and 60 ng of purified product used as template in DNA sequencing reactions. PCR products were sequenced using a Beckman-Coulter Dye Terminator sequencing Kit (see Chapter 3 for details) according to the standard protocols.

Sequence base-calling errors were corrected by manual inspection of electropherograms using the software program Bioedit (Hall 1999). The PCR gel electrophoresis data and the nucleotide sequence alignment of the rDNA-ITS and partial LSU-rDNA are shown in Appendices 2 and 3 respectively.

2.3.1. Alignment and Phylogenetic Analyses

DNA sequence data from *Chattonella* cf. *subsalsa* CHPI36 was aligned to comparable nucleotide sequences of other raphidophytes available from GenBank, using Clustal-X software (v.1.83, Jeanmougin *et al.* 1998), and improved by visual inspection. Details of the taxa included in the analyses are summarised in Tables 1 and 2.

Two sequence alignments were used to infer phylogenetic relationships among *Chattonella* spp. and the phylogenetic position of the *C.* cf. *subsalsa* (CHPI36). The rDNA-ITS dataset, contained 22 taxa and 730 characters in the sequence alignment. *Olisthodiscus luteus* (Ehrenberg) Reimann & Lewin was used as the outgroup for the analysis. The large subunit rDNA-LSU rRNA gene dataset contained 16 taxa and 1361 characters. The diatom *Cylindrotheca closterium* (Ehrenberg) Lewin & Reimann (syn. *Nitzschia closterium)* was used as an outgroup for the analysis. The LSU-rDNA sequences *Chattonella subsalsa* CCMP217 (AF409129) and *Chattonella* cf. *subsalsa* (CHPI36) were approximately1360 bp in length whereas all other sequences corresponded to approximately the first 680-700 bp of the alignment. Analyses were repeated with all sequences truncated to the first 700 bp of the alignment and any changes in branching order noted.

Table 1: Details of nucleotide sequences included in the phylogenetic analysis of partial LSU rDNA sequences

Species	GenBank Accession No.	Strain Code	Geographical locations
Chattonella* cf. *subsalsa	**Present study**	**CHPI36**	Pasabandar, south coast of Iran
Chattonella subsalsa	AF409126	CCMP217	Gulf of Mexico
Chattonella subsalsa	AF210736	CCMP217	Gulf of Mexico
Chattonella marina	AY704162		Hong Kong
Chattonella marina	AF210739	CCMP-217	Gulf of Mexico
Heterosigma akashiwo	AY704161		Hong Kong
Heterosigma akashiwo	AF086948	CCMP-452	Long Island Sound, USA
Heterosigma akashiwo	AF042820		Masan Bay, Korea
Heterosigma akashiwo	AF210741	CAWR05	
Vacuolaria virescens	AF210742	LB2236	
Vacuolaria virescens	AF409125	SAG1195.1	Wirral, Cheshire, England
Chattonella ovata	AF210738	NIES-603	Seto Inland Sea, Japan
Chattonella ovata	AY704163		Hong Kong
Chattonella antiqua	AF210737	NIES-1	Seto Inland Sea, Japan
Olisthodiscus luteus	AF210743	NIES-15	Seto Inland Sea, Japan
Cylindrotheca closterium	AF417666	K-520	

Table 2: List of species included in the phylogenetic analysis of ITS region of rDNA

Species	GenBank Accession No.	Strain Code	Geographical Locations
Chattonella* cf. *subsalsa	**present study**	**CHPI36**	Pasabandar, southeast coast of Iran
Chattonella subsalsa	AF409126	CCMP217	Gulf of Mexico
Chattonella subsalsa	AY858871	C. Tomas	Texas
Chattonella subsalsa	AY858870	C. Tomas	Singapore
Chattonella subsalsa	AY858869	C. Tomas	Sardinia
Chattonella subsalsa	AY858867	C. Tomas	Delaware
Chattonella subsalsa	AY858866	C. Tomas	California
Chattonella subsalsa	AY858864	C. Tomas	Japan
Chattonella subsalsa	AY858868	C. Tomas	North Carolina
Chattonella marina	AY858862	C. Tomas	North Carolina
Chattonella marina	AY858861	C. Tomas	Maryland
Chattonella marina	AY858860	C. Tomas	Japan
Chattonella marina	AY865604	CCMP 2049	Kagoshima Bay, Japan
Chattonella marina	AY704165		Hong Kong
Chattonella marina	AF137074	NIES 3	Osaka Bay, Japan
Heterosigma akashiwo	AY858874	CCMP 1680	Sandy Hook Bay, USA
Heterosigma akashiwo	AY858875	CCMP 1912	Kalaloch, USA
Vacuolaria virescens	AF409125	SAG1195.1	Wirral, Cheshire, England
Chattonella antiqua	AY858858	C. Tomas	Japan
Chattonella antiqua	AY858857	CCMP 2052	Mikawa Bay, Japan
Chattonella antiqua	AY858856	CCMP 2050	Seto Inland Sea, Japan
Chattonella antiqua	AF136761	NIES 1	Seto Inland Sea, Japan
Chattonella ovata	AY858872	CCMP 216	Japan
Chattonella ovata	AY858863	C. Tomas	Japan
Chattonella ovata	AY704166		Hong Kong
Fibrocapsa japonica	AF112991	LB 2162	
Olisthodiscus luteus	AF112992	NIES-15	Seto Inland Sea, Japan

PAUP* version 4.0b10 for Macintosh (PPC) was used (Swofford 2002) for all phylogenetic analysis of rDNA-ITS region and partial LSU rDNA. Phylogenetic structure was examined and tested by the randomisation tests and probability tables of critical values of g_1 (Hillis & Huelsenbeck 1992). Neighbour-joining (NJ) trees were constructed with the minimum evolutionary (ME) model using LogDet distances (Bolch & Campbell 2004) (ME-LgD) and the mean distance metric. Maximum parsimony (MP) analyses used the branch and bound search algorithm to find the most parsimonious trees. All characters were equally weighted, gaps were treated as missing data; multistate characters were interpreted as uncertainty. To

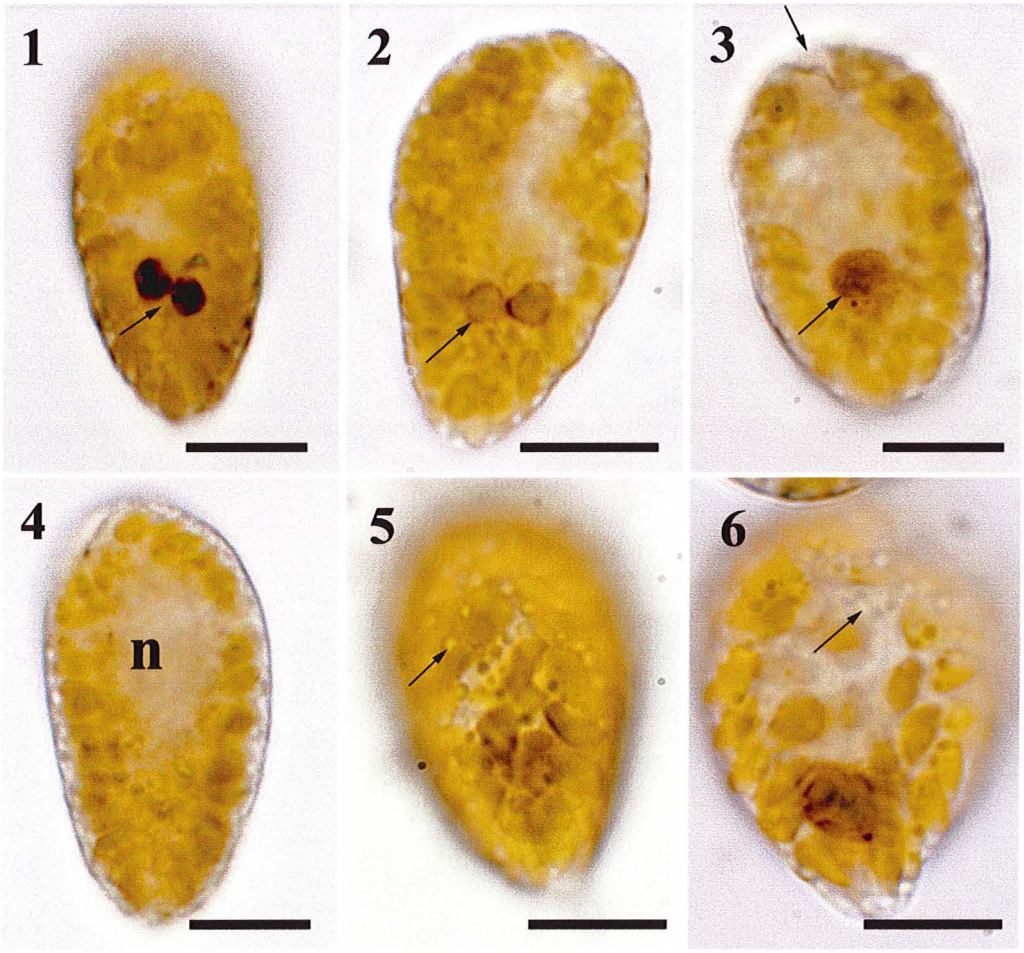
estimate the reliability of the MP trees and the NJ tree, bootstrap analyses were carried out utilising 100 replicates of the full heuristic search algorithm.

3. Results

3.1. Morphology

Cells of *Chattonella* cf. *subsalsa* CHPI36 are 24-43μm in length and 17-23μm wide, slightly compressed, and tear-shaped to lanceolate in lateral view (Figs 1-3 & 7). Some cells show a posterior tail (Figs 8 & 9). Two sub-equal flagella project from two flagella grooves that arise from a clearly defined anterior depression (Figs 3, 10 & 11). The cells appear golden-yellow in colour under bright field illumination. The numerous densely packed chloroplasts are green, peripherally placed, and ellipsoid to ribbon shaped (Figs 7 & 9). The large oval-shaped nucleus extends from beneath the anterior depression of the cell toward the cell centre (Figs 4 & 8). Numerous mucocysts are present on the cell surface (Figs 5 & 6). Two dark brown-red eyespots are present (Figs 1 & 12). Examination of live cells under the light microscope causes cells to quickly lose motility and the eyespot colour fades to a lighter brown (Fig. 2). The eyespot is not visible in lateral view (Fig. 4). The chloroplasts are green in early growth phase, however, in older cultures darker green the chloroplasts are dark-green to green-brown.

Presumed encystment was observed in late stationary-phase cultures growing in nutrient depleted media approximately 6 months after transfer. Cysts were not produced in cultures transferred into nutrient-deficient media and after few weeks all cells died. When grown in nutrient-replete medium, non-motile spherical cells ranging from 9-12μm in diameter was formed in older stationary-phase cultures (Figs 1, 2, 17 & 18). The putative resting cysts are pale-brown and spherical, ranging from 17-21μm in diameter. A large brown accumulation body was observed in the resting cysts (Figs 13-16).



Figs 1-6. LM. Motile cells of *Chattonella* cf. *subsalsa* CHPI36, germinated from mixed incubated sediment.

Fig. 1. Cell showing two dark brown eyespots.

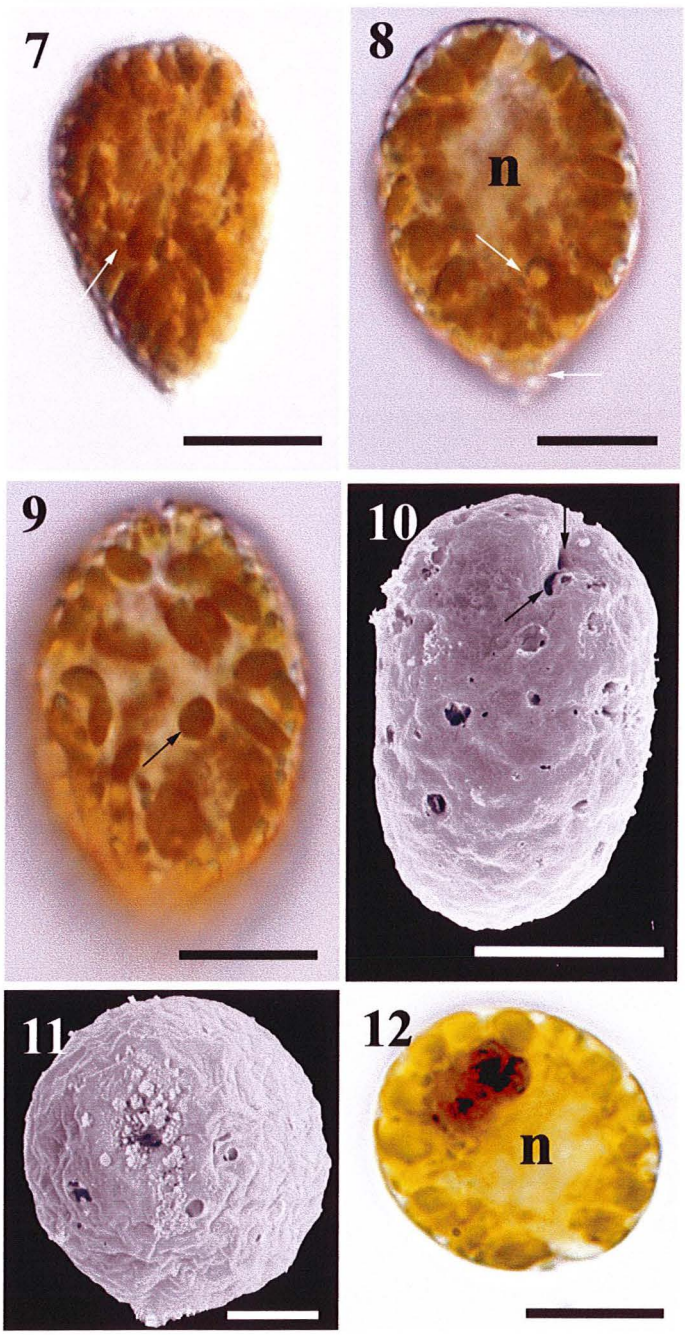
Fig. 2. Same cell as Fig. 1. Note the eyespot colour changes to a lighter colour during observation.

Fig. 3. Same cell in lateral view, showing one visible eyespot (bottom arrow). Note anterior depression (top arrow).

Fig. 4. Cell showing a large oval nucleus.

Figs. 5-6. Surface focus of cell showing mucocysts on the surface (arrow).

All scale bars=10µm.



Figs 7-12. *Chattonella* cf. *subsalsa*, vegetative cell.

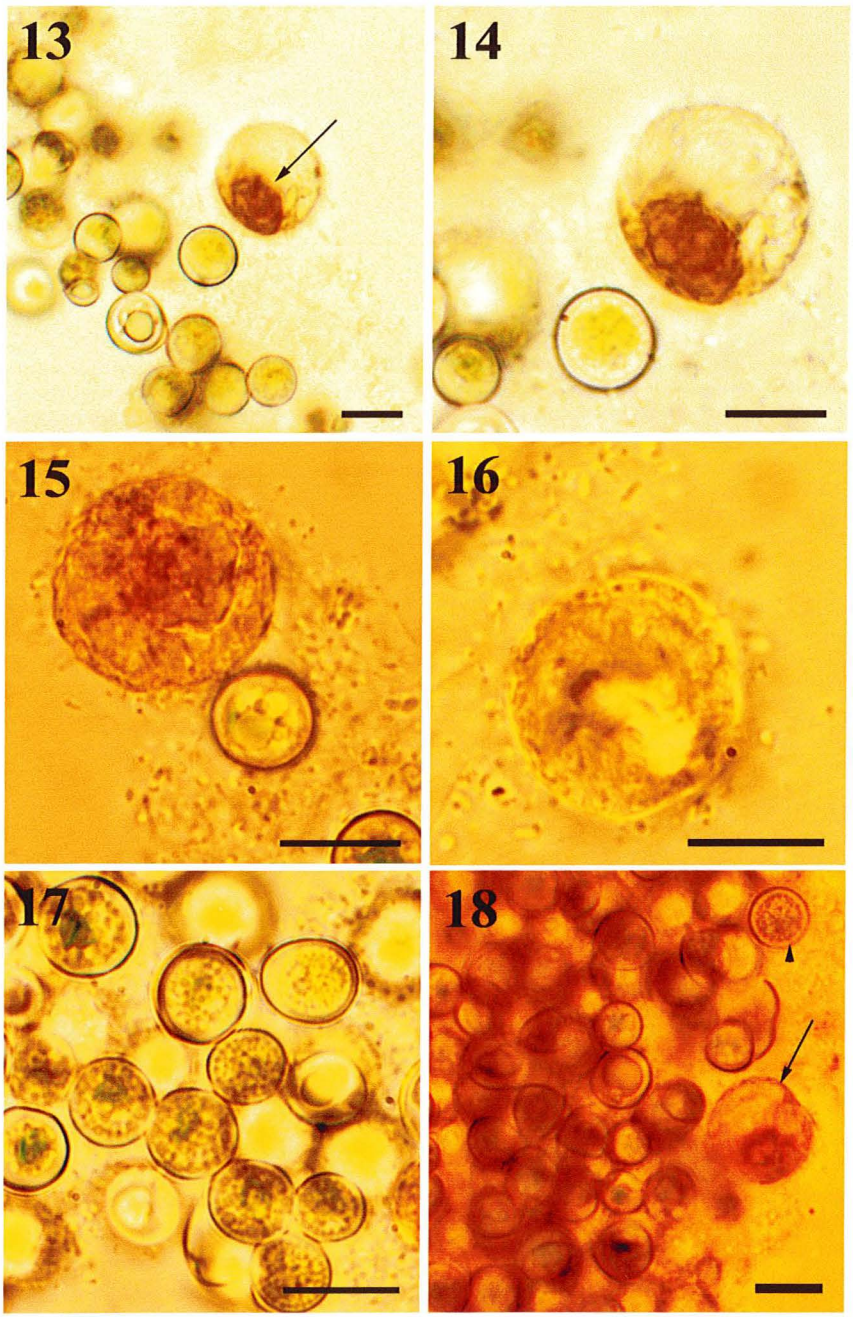
Fig. 7. LM. Tear-shaped cell showing the densely-packed chloroplasts (arrow).

Fig. 8-9. LM. Cell showing a posterior tail (bottom arrow). Note the nucleus (n)and eyespot (top arrow).

Fig. 10-11. SEM. Cell showing the anterior depression and two flagella grooves (arrows).

Fig. 12. Polar view of cell showing the nucleus (n) and eyespot.

All scale bars=10µm except Fig. 11=5µm.



Figs 13-18. LM. *Chattonella* cf. *subsalsa* putative resting cysts and non-motile cells.
Figs 13-14. Spherical cyst showing large accumulation body (arrow). Note non-motile cells, produced in old cultures.
Figs 15-16. Cysts of *C.* cf. *subsalsa* surrounded with a mucilaginous layer
Figs 17-18. Non-motile spherical cells produced in nutrient depleted medium. Note the size of cyst (arrow) compared to typical non-motile cells (arrow head).
All scale bars=10μm.

3.2. Phylogenetic Analyses

Significant phylogenetic structure was found in both datasets ($P<0.01$). All random tree distributions were negatively skewed. For the rDNA- ITS data (g1= -1.449 for NJ, LogDet g1= -1.444 for MP). For the partial LSU-rDNA (dataset g1= -1.176 for NJ mean distance, g1= - 0.82 for MP).

For the partial LSU-rDNA data both the NJ and MP analyses generated a tree with the same primary branching order (see Figs 19 & 20). All analyses show that the included raphidophyte taxa form a monophyletic group with 100% bootstrap support. In both trees, *Olisthodiscus luteus* branches first followed by *Vacuolaria* and finally *Chattonella*. The genus *Chattonella* is monophyletic in both trees.

Within *Chattonella*, two monophyletic groups were formed, one comprising all strains of *Chattonella marina*, *Chattonella ovata*, *Chattonella antiqua* with 100% bootstrap support that are virtually identical (referred to as *C. marina* group hereafter), differing by only 1-2 nucleotide substitutions. The two *Chattonella subsalsa* sequences are also virtually identical. *Chattonella* cf. *subsalsa* CHPI36 clusters with *C. subsalsa* but is clearly distinct, differing by 17 base-pairs over the 1372 bp of LSU-rDNA examined.

The trees derived from analysis of rDNA-ITS sequences support the analyses of the LSU-rDNA data. Both NJ and MP analyses of the rDNA-ITS resulted in a trees with similar branch order (Figs 21 & 22). MP analysis resulted in two most parsimonious trees with identical branching order, therefore only one is presented in Fig. 21. In this tree the branches orders are *Fibrocapsa japonica*, *Vacuolaria virescens* (freshwater species), *Heterosigma akashiwo,* followed by *Chattonella* spp. respectively. Within *Chattonella*, two clades were formed as in the LSU analysis. The first clade comprised the *C. marina* group that are virtually identical (1-4 nucleotides difference) across the rDNA-ITS. The second clade comprised all *Chattonella subsalsa* strains and *Chattonella* cf. *subsalsa* CHPI36. Strain CHPI36 again is clearly distinct from all other *C. subsalsa* in the analysis, differing by 12 base-pairs over the ITS- region.

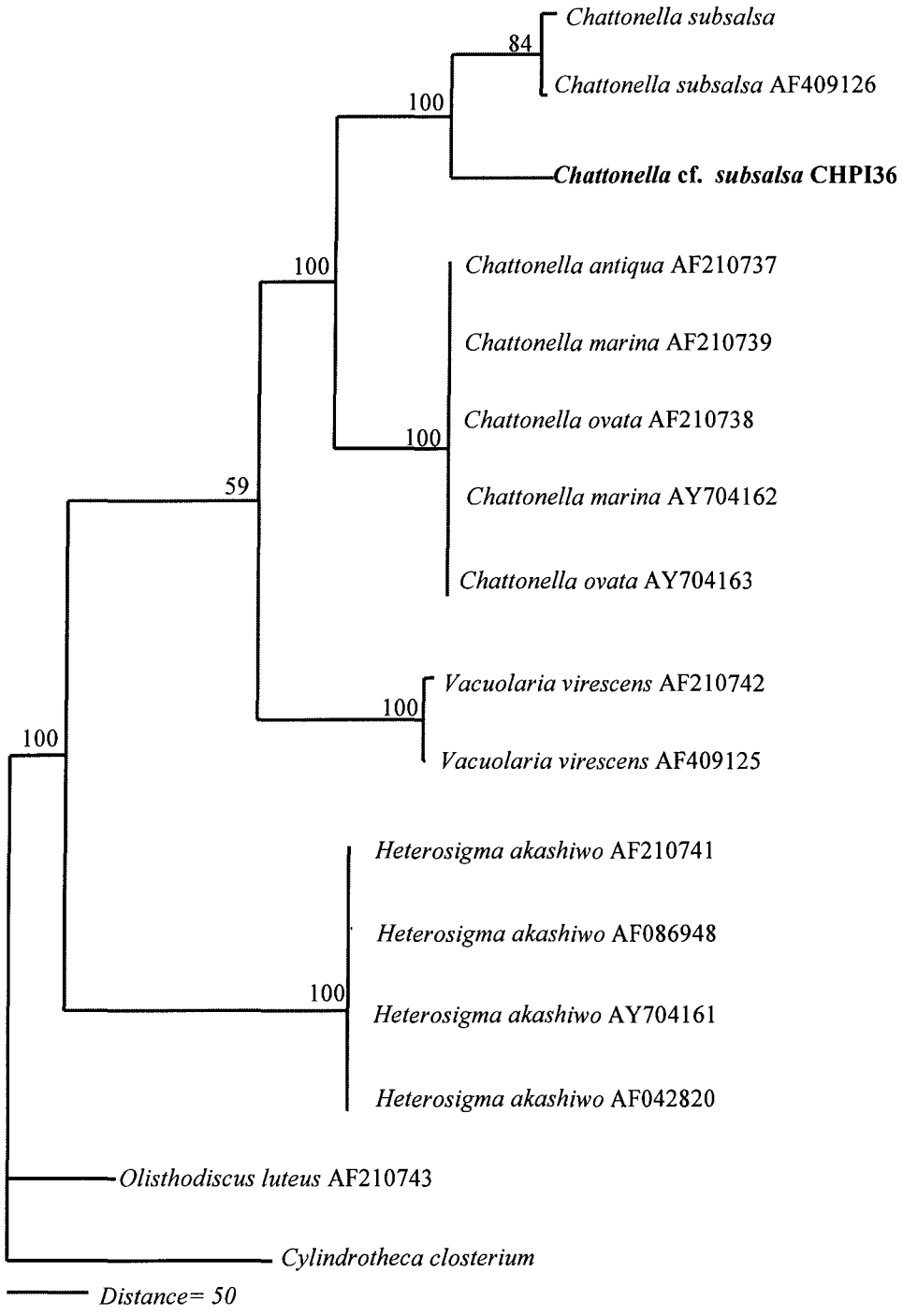


Fig. 19. Phylogenetic relationship among Chattonellate raphidophytes inferred from phylogenetic of partial LSU of rDNA gene. Most parsimonious tree obtained using branch and bound search. Bootstrap values from 100 replicates are shown above the nodes. *Cylindrotheca closterium* is the outgroup taxon.

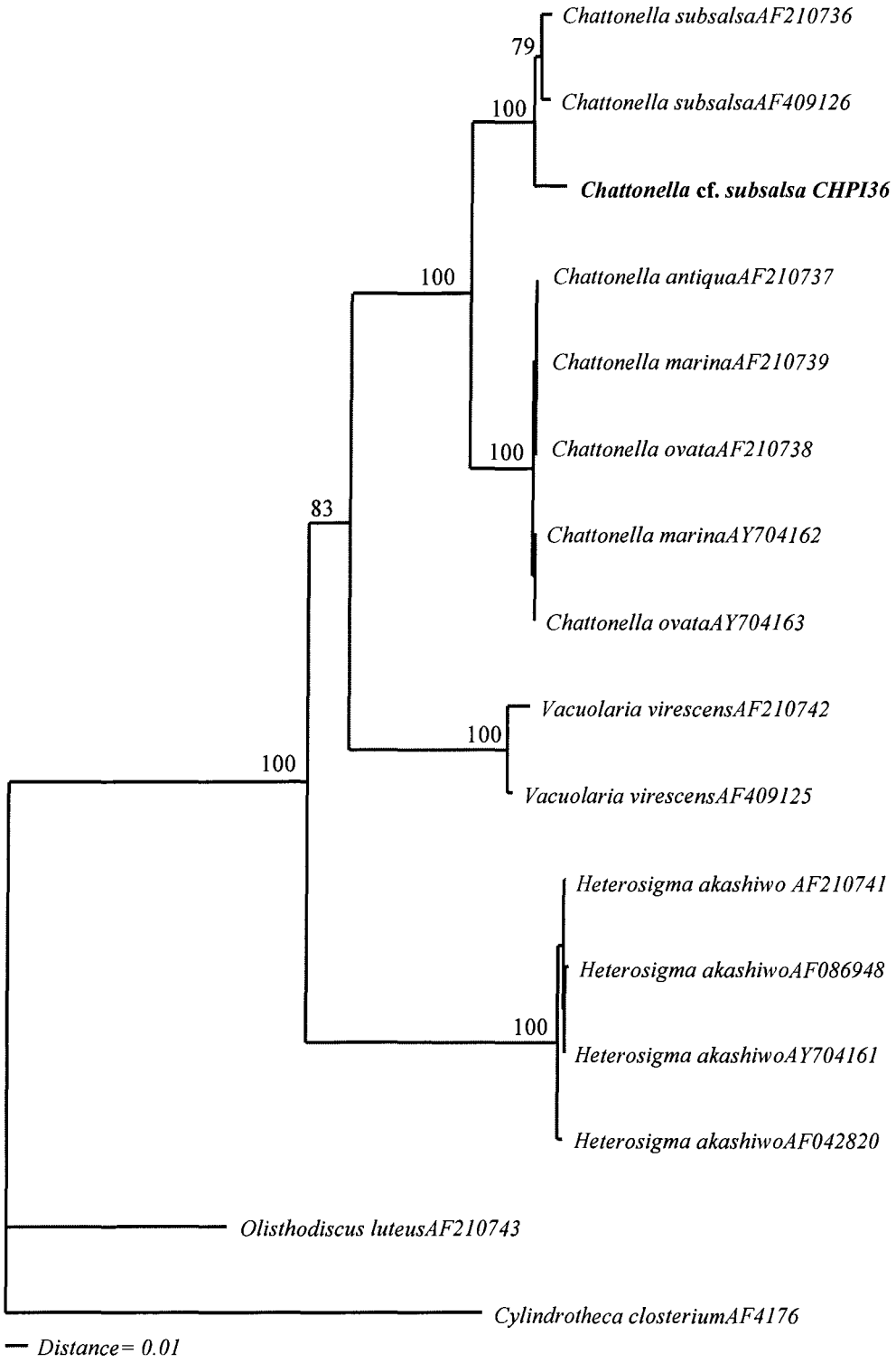


Fig. 20. Phylogenetic relationship among Chattonellate raphidophytes based on partial LSU rDNA gene sequences (domains D1-D3). Neighbour joining (NJ) tree was produced from PAUP mean distances. The numbers above branches show bootstrap support (100 replicates) for each cluster. *Cylindrotheca closterium* was used an outgroup taxon.

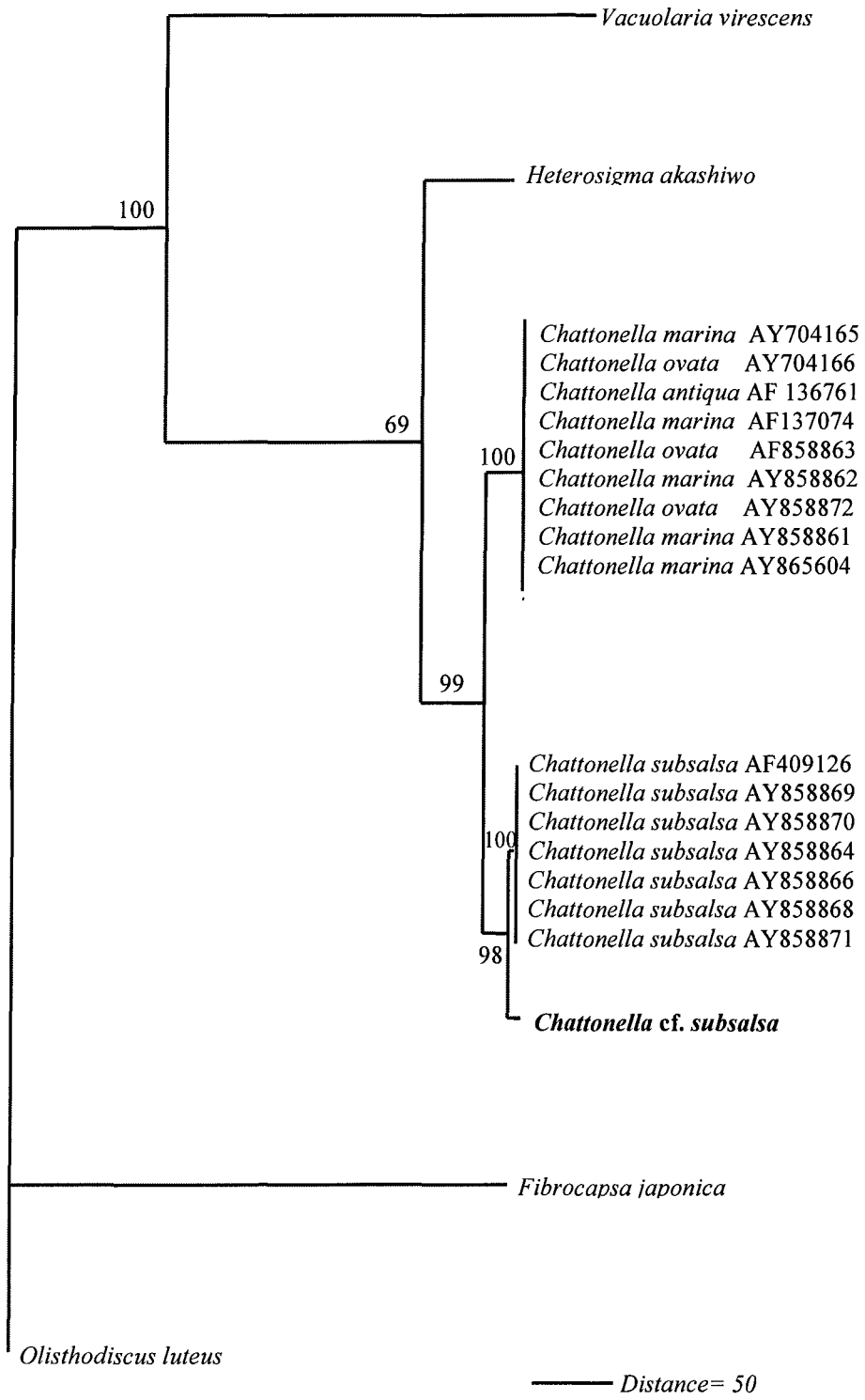


Fig. 21. Relationship of *Chattonella* cf. *subsalsa* CHPI36 to other Chattonellate raphidophytes inferred from the phylogeny analysis of ITS regions of rDNA gene. Most parsimonious tree obtained using branch and bound search. Values above nodes represent bootstrap values (100 replicates). *Olisthodiscus luteus* is outgroup taxon.

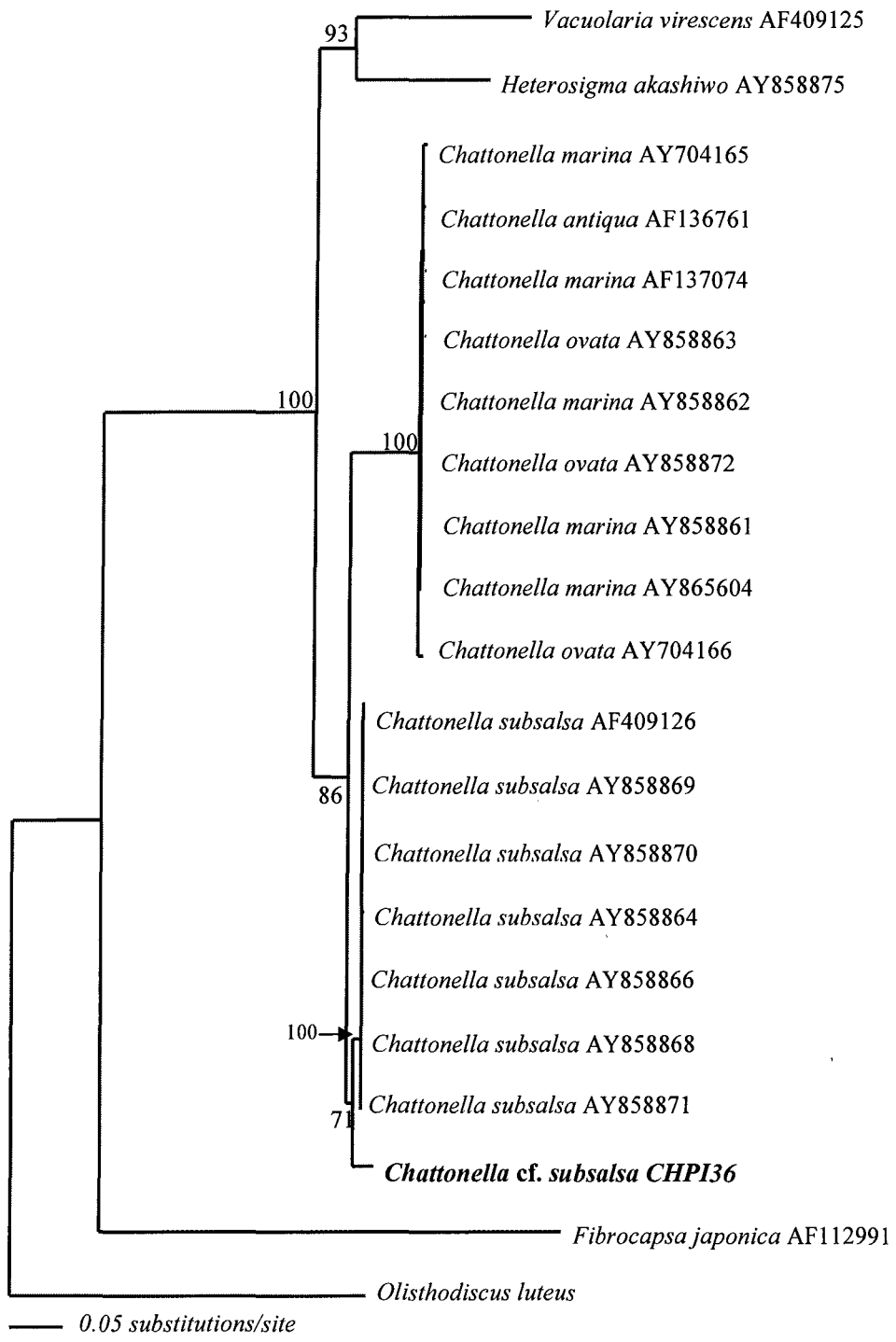


Fig. 22. Relationship of *Chattonella* cf. *subsalsa* CHPI36 to other Chattonellate raphidophytes species inferred from the phylogenetic analysis of the ITS regions of rDNA gene. The tree was constructed by neighbour-joining from Log-Det genetic distance (ME-LgD analysis). Numbers above branches represent bootstrap support values (100 replicates). *Olisthodiscus luteus* was used as an outgroup taxon.

4. Discussion

4.1. Morphology of *Chattonella* cf. *subsalsa* CHPI36

Due to the morphological similarity between *C. subsalsa* and *C. marina,* identification based on light microscopy is often difficult (Fig. 23). *C. subsalsa* is the type species for the genus and morphologically related to *C. marina.* Hara and Chihara (1982) separated these two species based on two ultrastructure characteristics: the presence of oboe-shaped mucocysts in *C. subsalsa* and also the relationship between the thylakoid membranes and chloroplast pyrenoid matrix. In *C. subsalsa,* the thylakoids do not penetrate the pyrenoid, but in *C. marina* the thylakoids are in the pyrenoid matrix, and the cells have distinctive mucocysts. However, there are a number of unresolved questions regarding the type material of *C. subsalsa,* and it has been suggested re-examination of cells from the type locality is necessary to clarify the identity and circumscription of both *C. subsalsa* and *C. marina* (Hallegraeff and Hara 2003).

In the present study, the cell shape of *C.* cf. *subsalsa* CHPI36 resembles *C. marina* more than *C. subsalsa,* sometimes possessing a posterior tail similar to *C. marina.* Compared with *Chattonella subsalsa* CCMP217 (Figs 24-29), the two strains have quite different cell outlines, however, cell shape is known to be pleomorphic in most Chattonellate raphidophytes (Tomas 1987; Hara & Chihara 1987; Aizdaicher 1993). For example, older cultures of *C. marina* become narrower and longer similar to *C. antiqua* (Band-Schmidt *et al.* 2004) suggesting that cell morphology alone is not a reliable taxonomic character.

Chattonella cf. *subsalsa* CHPI36 clearly differs from *C. antiqua* due its smaller size (*C. antiqua* 70-130μm long), the lack of long posterior tail, and the presence of mucocysts in the cell surface (Table 3).

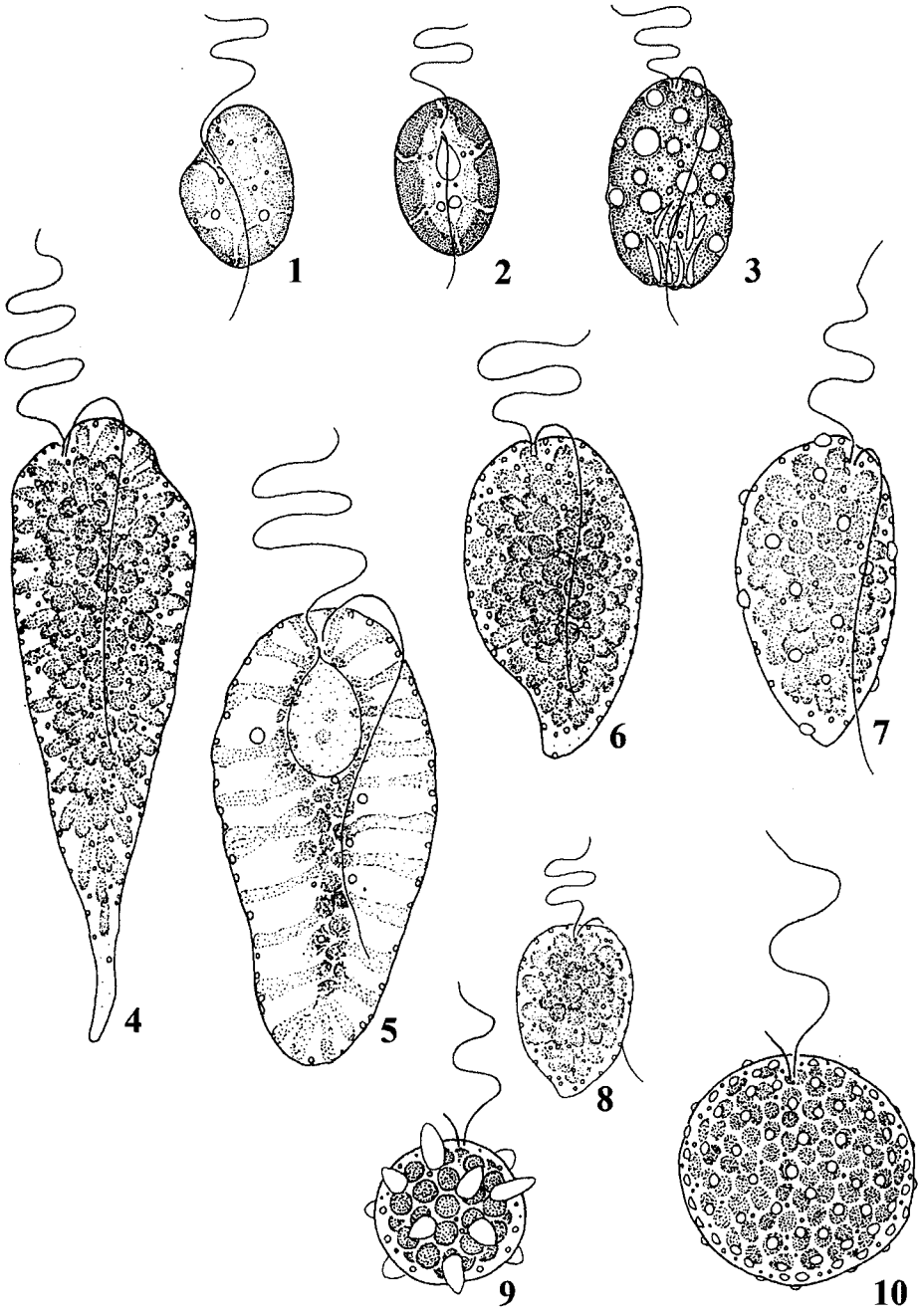
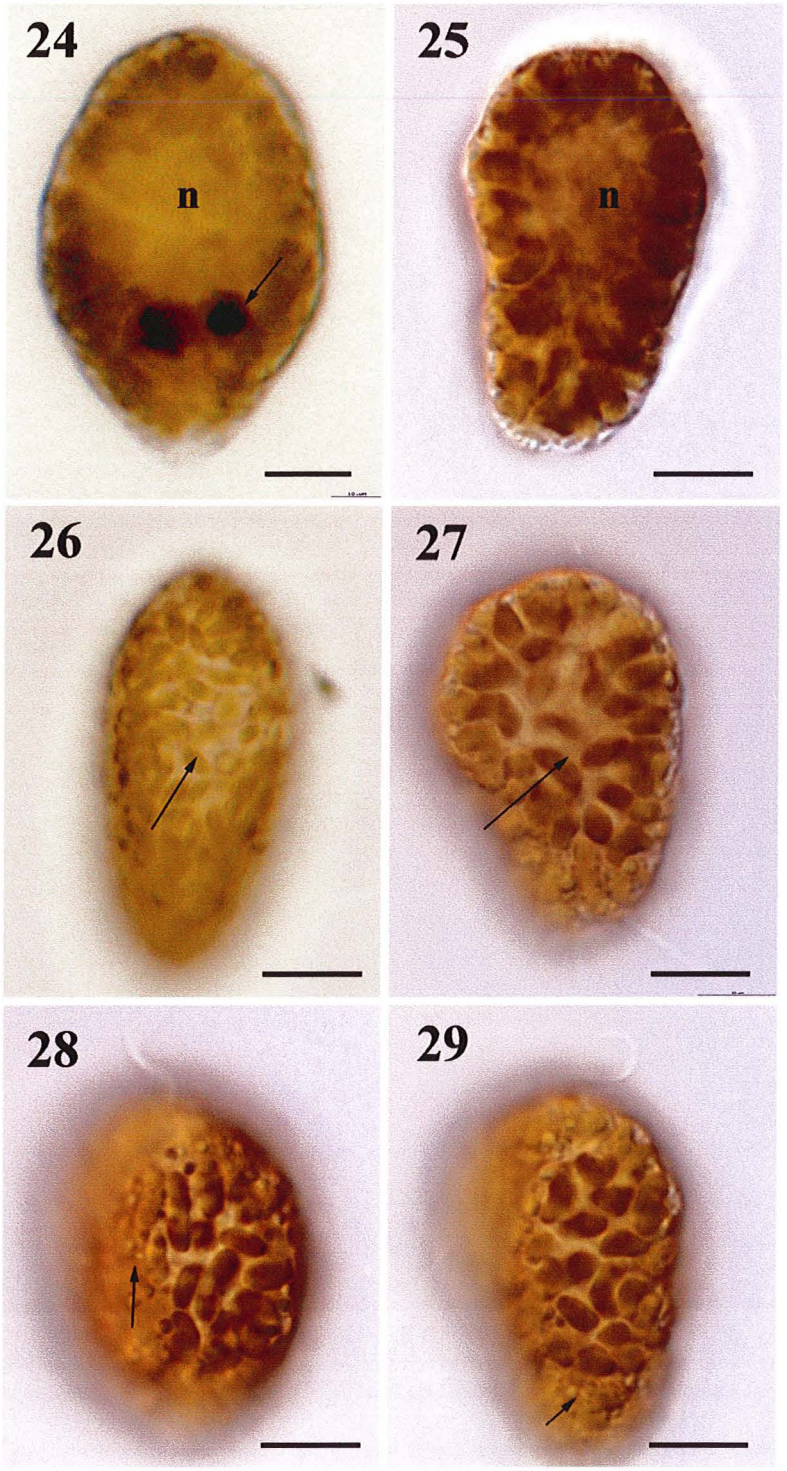


Fig. 23. Morphological comparison of Chattonellate species.1. *Heterosigma carterae*; 2. *Olisthodiscus luteus*, 3. *Fibrocapsa japonica*; 4. *Chattonella antiqua*; 5. *Chattonella ovata*; 6. *Chattonella marina*; 7. *Chattonella subsalsa*; 8.*Chattonella minima*; 9. *Chattonella verruculosa*; and 10. *Chattonella globosa.* (from Hallegraeff & Hara 2003).



Figs 24-29. LM. Comparison of the morphology of vegetative cells of *Chattonella* cf. *subsalsa* CHPI36 with *C. subsalsa* CCMP217.

Fig. 24. Strain CHPI36 in deep focus. Note the presence of two eyespots.

Fig. 25. CCMP217 in deep focus. Note the lack of eyespot.

Figs 26-27. Strains CHPI36 (left) and CCMP217 (right) in surface focus showing chloroplast shape and arrangement.

Figs 28-29. Strains CHPI36 (left) and CCMP217 (right). Note the presence of mucocysts (arrow).

All scale bars=10μm.

Table 3. Comparison of *C.* cf. *subsalsa* CHPI36 with similar *Chattonella* spp.

Vegetative cell	*C.* cf. *subsalsa* CHPI36	[a]*C. subsalsa*	[b]*C. marina*	[c]*C. antiqua*
Size: length µm	24-43	30-50	30-70	70-130
Width µm	17-23	15-25	20-30	30-70
Chloroplast: colour	Green-brown	Green-brown	Yellowsh-Green-brown	Green-brown
shape	ellipsoid	ellipsoid	ellipsoid	Ellipsoid to tear-shaped
eyespot	present	absent	absent	absent

[(a,b, c)] described in Hallegraeff & Hara (2003)

Chattonella cf. *subsalsa* CHPI36 resembles *C. subsalsa* in many features: Both possess a tear-shaped nucleus that centrally positioned; both have oval-shaped chloroplasts that are peripherally placed; both have possess many mucocysts on the cell surface, and neither possess contractile vacuoles. The anterior depression where flagella arise is deep and clear in *C.* cf. *subsalsa* CHPI36, however, this feature is not clearly documented for *C. subsalsa* (Hallegraeff and Hara 2003). Some species in the genus *Chattonella* possess a shallow anterior depression such as *Chattonella globosa* Hara & Chihara (Hara *et al.* 1994).

Strains of *C. ovata* resemble *C. antiqua*, but contractile vacuoles are present, and Hallegraeff and Hara (2003) suggest that *C. ovata* may be an ecotype of *C. antiqua.* The similarity of these two species agrees with molecular analyses presented here.

There is little information on the resting cysts of *C. subsalsa* and those that refer to the production of a resting stage do not give morphological descriptions (e.g. Steidinger & Penta 1999). The putative cysts of *C.* cf. *subsalsa* CHPI36 are smaller than the yellow-greenish to brownish, hemispherical cysts described for *Chattonella marina* (20-30µm diameter; Imai 1989). The large dark brown accumulation body in cysts of *C.* cf. *subsalsa* CHPI36 also differs from the several dark brown spots or black material in the cysts of *C. marina* (Imai 1989).

The appearance of small cells (before encystment) in N-limited medium has been reported for *C. marina* (Imai 1989), and Band-Schmidt *et al.* (2004) noticed that the

morphology of *C. marina* is affected by the age of culture. In older and also N-limited cultures, cells become smaller, more ovoid or spherical and non-motile. Similar changes were noted in nutrient limited cultures of *C.* cf. *sabsalsa* CHPI36.

Strain CHPI36 is similar in general morphology to *C. subsalsa* CCMP217 as both have similar chloroplast arrangement, cell shapes and mucocysts. Despite these similarities, *C.* cf. *subsalsa* CHPI36 and *C. subsalsa* show distinct differences. The cell shape/outline of strain CHPI36 is different (Figs 24-29) and it is slightly smaller than *C. subsalsa*, although there is considerable overlap in the size ranges (Hallegraeff & Hara 2003) (Table 3). Strains of *C.* cf. *subsalsa* CHPI36 also have an obvious eyespot, whereas *C. subsalsa* and most other members of chattonellates do not possess an eyespot (e.g. *H. akashiwo, O. luteus, F. japonica, C. antiqua, C. marina*; Hara & Chihara 1982; Hallegraeff & Hara 2003). Colour is also considered as one of the important feature in distinguishing *C. subsalsa* from *C. marina* (Hallegraeff & Hara 2003). *C. subsalsa* possesses a green-brown colour, whereas *C. marina* possesses a green yellowish-brown colour. Although colour can be a somewhat subjective character, under identical culture conditions, *C.* cf. *subsalsa* CHPI36 differs from *C. subsalsa* by having a more green colour.

4.2. Molecular Analyses

The analyses presented here show that the raphidophytes included here form a monophyletic group with high bootstrap support that includes both the marine and freshwater (*Vacuolaria*) genera. This agrees with previous studies (Daugbjerg & Anderson 1997; Potter *et al.* 1997; Ben Ali *et al.* 2001; Ben Ali *et al.* 2002). Members of the four different genera are quite distinct from each other. Connell (2000) suggested that *Heterosigma akashiwo* ITS sequence was quite divergent from both *C. antiqua* and *C. subsalsa* (20 and 18% divergence respectively) and the present study supports this result. The two strains of *Heterosigma akashiwo* were distinct from all members of other genera and with sequence divergence of 22 and 19% in ITS sequence from the two later species. In addition, *Fibrocapsa japonica* and *Olithsodiscus luteus* showed high ITS sequence divergence by pairwise comparison (47%, Connell 2000; 50%, present study). The amount of either ITS

sequence or partial LSU sequence divergence and nucleotide base difference between *Chattonella* species and other species of different genera is high.

Many algae species show sequence variation in the rDNA-ITS and LSU-rDNA genes among geographical isolates within the same species (Chopin *et al.* 1996; Bolch *et al.* 1998; Hirashita *et al.* 2000). For example, Atlantic and Pacific isolates of *Cladophora albida* showed up to 1% sequence divergence across the ITS region with each oceanic basin and as much as 21% between the two oceanic basins (Bakker *et al.* 1992). *Peseudo-nitzchia delicatissima* also showed high rDNA-ITS diversity among different strains isolated at different phases of blooms from same geographical area (Orsini *et al.* 2004). In contrast, past studies on raphidophytes demonstrated little or no variation of LSU-rDNA and rDNA-ITS sequences within each species (e.g. Connell 2000, Hirashita *et al.* 2000; Connell 2002). For example, 20 strains of *Heterosigma akashiwo* from across the globe have almost 100% ITS sequence identity, indicating that populations of this species represent only one worldwide species (Connell 2000). Similar results have been reported for 16 isolates of *Fibrocapsa japonica* (Kooistra *et al.* 2001). The sequences from four *Heterosigma akashiwo* used in the present study were also virtually identical across the 700 bp of LSU examined, with the sequence divergence of less than 0.6% between strains.

Within the genus *Chattonella*, strains of *C. marina*, *C. ovata* and *C. antiqua* show remarkable similarity across the rDNA-ITS regions with <1.2% sequence divergence between *C. marina and C. ovata* and only few bases (max. 7 nucleotides) differences in nucleotide sequence. Past studies of *Chattonella marina* and *C. antiqua* have considered these to be one species (Connell 2000; Hirashita *et al.* 2000; Sako *et al.* 2000; Connell 2002). From this study and previous work, it is clear that global geographical variation in both the LSU-rDNA and rDNA-ITS is very low within raphidophytes. *Chattonella subsalsa* is a sister taxon to the *C. marina* group from the analyses presented here.

While all eight *C. subsalsa* strains are identical across rDNA-ITS, strain CHPI36 from the southeast coast of Iran is clearly distinct from the others (2.6% sequence divergence across 575 nucleotide positions). The LSU-rDNA data also support the distinctiveness of strain CHPI36 from *C. subsalsa*. Two available sequences of

Chattonella subsalsa (AF210736, AF409126) are also nearly identical (0.8% sequence divergence over 1372 bp), yet *C.* cf. *subsalsa* CHPI36 is clearly distinct from these *C. subsalsa* strains. In light of the highly conserved nature of rDNA within *C. subsalsa* and raphidophytes generally, and the small but consistent morphological differences to *C. subsalsa*, these findings indicate that strain CHPI36 is either a sub-species of *C. subsalsa*, or more likely a distinct species related to *C. subsalsa.*

5. Conclusions

Chattonella cf. *subsalsa* CHPI36 is morphologically very similar to, but genetically distinct from *Chattonella subsalsa.* Comparative analyses of LSU-rDNA and rDNA-ITS sequences of *C. subsalsa* strains from different geographical areas worldwide show that *C. subsalsa* (and other Chattonellates) show little or no genetic variation and that *C.* cf. *subsalsa* CHPI36 is a unique genotype related to *C. subsalsa.* The morphological and molecular analyses presented here strongly suggest that CHPI36 is either a subspecies of *C. subsalsa* or a new species of *Chattonella.* However, at this stage further investigation, such as ultrastructural studies using TEM, is required to be able to justify the description of a new species.

6. Further study

The ultrastructure of *Chattonella* cf. *subsalsa* CHPI36 should be investigated and compared with that of the type species *Chattonella subsalsa* (Mignot 1976). In addition, investigation of the life cycle including cyst stage of this species may help to understand the true affinity of the species. The role of this species and other raphidophytes in bloom initiation or termination should be investigated along the southeast coast of Iran, where it is associated with annual blooms and occasional fish-kills. Factors such as light intensity, salinity and temperature that may affect the ecology of the species in the natural environment may help predict blooms. As raphidophytes cysts stick to solid surface such as diatom frustules and sand grains (Imai & Itakura 1999), their presence may go unnoticed in dinoflagellate cyst surveys. Therefore, a specific raphidophyte cyst survey should be considered as

many raphidophytes are toxic, potentially harmful species that cause serious problems for aquaculture.

7. References

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CHAPTER 6

Discussion and Summary

The present investigation is the first record and description of the dinoflagellate and raphidophyte cyst flora from recent marine sediments of the southeast coast of Iran (the Oman Gulf in the Indian Ocean). This study was an attempt to significantly increase the knowledge of the cyst flora in a tropical region. Dinoflagellate cysts of the tropical and subtropical areas are poorly described compared with temperate regions, especially in coastal waters. In addition to the morphological analyses of the cysts, germination and molecular studies have also been carried out. The main findings of this study can be synthesised and summarised as following.

The assemblage of dinoflagellate cysts in the study area is characterised by an overall high proportion of calcareous peridinioid cysts. Past studies on dinoflagellate cyst assemblage of open ocean waters have demonstrated that calcareous dinoflagellate cysts are main components of the cyst flora in the tropical and subtropical areas (e.g. Dale 1992; Holl *et al.* 1998, 1999; Karwath *et al.* 2000). Additionally, the studies of Dale (1992) in sediment trap material also shows a high abundance of calcareous cysts in the Equatorial Pacific and Atlantic Oceans. On the other hand, this group not only composes a higher proportion in offshore areas, but also is an important component of the coastal area. Moreover, several reports from temperate coastal sediments have demonstrated that calcareous resting cysts can dominate (e.g. Ellegaard *et al.* 1994; Nehring 1994; Blanco 1995).

In Iranian coastal sediments, *Scrippsiella trochoidea* and *Scrippsiella* sp. 2 are the most common cyst types among calcareous dinoflagellate cysts; *Scrippsiella lachrymosa*, *Scrippsiella irregularis* sp. nov., *Scrippsiella* sp. 3 and *Scrippsiella* sp. 4

were found in lower concentrations. Due to its relatively short mandatory dormancy (Binder & Anderson 1987), *S. trochoidea* has capability of rapid shifting from the motile cell to the cyst and therefore giving constant inoculation of cells over several months in a highly variable environment (Montresor *et al.* 1998). In the Arabian Sea, where monsoon currents provide a highly variable oceanography, different types of spiny cysts, all attributed to the *S. trochoidea*-like complex, are reported by Wendler *et al.* (2002a). Some other calcareous cysts such as *Calciodinellum levantinum*, *Calciodinellum albatrosianum* and *Leonella granifera* have been recorded from offshore waters of Arabian Sea (Wendler *et al.* 2002b; Vink 2004). In this work, the Pasabandar coastal area possessed more *S. trochoidea* than the estuary and bay environments of Bahoo-Kalat and Chabahar (see Chapter 2 for more details).

Compared with other tropical or subtropical coastal cyst assemblages from the southwest India or the Gulf of California (Godhe *et al.* 2000; Morquecho & Lechuga-Dveze 2003), the Iranian assemblages contain much lower and less consistent occurrence of potentially toxic species such as *Alexandrium* spp. or *Lingulodinium polyedrum*. The cyst assemblage of the subtropical Gulf of California also contains cyst of *Gymnodinium catenatum* whereas, this was not found from Iranian coastal sediments. However, its occurrence in the tropical area of the Arabian Sea is often related to the presence of upwelling (Marret & Zonneveld 2003). All these tropical areas contain high concentrations and more consistent occurrences of *Scrippsiella trochoidea.*

It has been documented that specific cyst assemblages are associated with a specific depth and climate conditions (Wall *et al.* 1977). Some species such as *Spiniferites elongatus* Reid and *Scrippsiella trifida* Lewis are restricted to temperate areas (Joyce 2004; Head *et al.* 2006) and some such as *Bitectatodinium spongium* Zonneveld & Jurkschat and *Pyrodinium bahamense* are restricted to the tropical to subtropical areas (Zonneveld & Jurkschat 1999; Azanza *et al.* 2004).

In tropical areas such as Arabian Sea, South China Sea and Malampaya Sound coast in Philippines, the cyst assemblage is typically affected by monsoon currents (Zonneveld 1997; Borja *et al.* 2000; Kawamura 2004). Fig. 1 shows the general pattern of monsoon wind and water circulation in the Arabian Sea. A high nutrient

concentration is associated with the NE monsoon along the Iranian coast (Zonneveld 1997). Iranian cyst assemblages are possibly affected by SW or NE monsoon as discussed in Chapter 2. Some species such as *Protoperidinium monospinum*, *Protoperidinium compressum Pentapharosdinium dalei* have been reported in high relative abundance from Pakistan and Oman coastal sediments (adjacent area, see Fig. 1) (Zonneveld 1997), but are totally absent from Iranian coastal sediment. Relative abundance of these species is correlated to SW or NE monsoon currents (Zonneveld 1997). Absence of a typical warm water species of *Pyrodinium bahamense* has also been discussed in Chapter 2.

Although some possible factors that affect the relative abundance of the cyst flora in different locations of the present study have been discussed in Chapter 2, it is difficult to interpret which ecological factors (particularly which monsoon current) affect abundance of each taxon in Iranian coastal regions. Further ecological investigations are required. However, it is clear from this study that there are a number of new species in this tropical area and their distribution pattern needs to be assessed (for example, different types of unknown *Protoperidinium* species). *Protoperidinium* was found to be the most diverse group with 20 cyst types identified in the study area. Of these, 8 species remain unknown and may be new species; however, without germination and plate pattern observation, it is premature to introduce them as new species. For example, cyst of *Protoperidinium* sp. 1 is quite similar to the cyst of *P. oblongum*, but germination of both cysts produced different species. *Protoperidinium* sp. 1 is possibly a new species that possesses a different archeopyle shape and distinct motile cells (see Chapter 2). But only one cyst was germinated and its thecal plate pattern was not clear by light microscopy. In addition, many smooth wall pentagonal cyst types were found that resembled *P. oblongum* cysts but, these may also be different species that need to be confirmed by germination. Moreover, three *Protoperidinium pentagonum* –like cysts were identified in Iranian sediments and, to our knowledge, one of them (type three) has not previously been described. As the germination of the cyst was unsuccessful, it remains as another type of *P. pentagonum.*

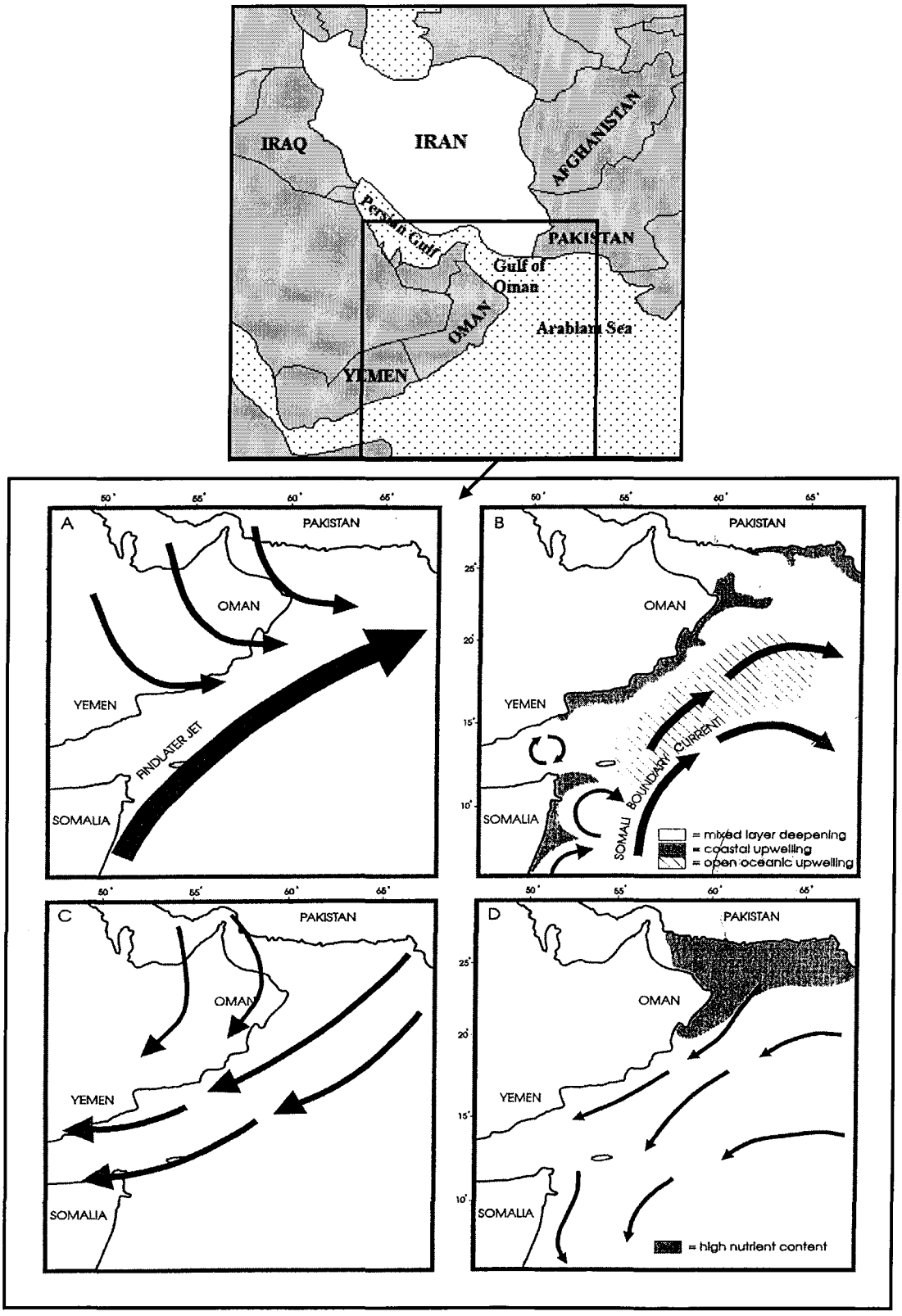

Fig. 1. General pattern of monsoon wind and water circulation in Arabian Sea; (A) General wind pattern during the SW monsoon; (B) General surface-water circulation during the SW monsoon; (C) General wind pattern during the NE monsoon; (D) General surface-water circulation during the NE monsoon (from Zonneveld 1997).

Another unique and novel species is microreticulate trapezoidal cyst that was found in this study. This new species (*Gymnodinium trapeziforme*) is naked, non-chain-forming and non-toxic. Its affinity with other microreticulate cyst-producing gymnodinioids was confirmed by germination and molecular studies. Phylogeny of the gymnodinioid-like species is in accordance with previous studies (e.g. Daugbjerg *et al.* 2000; de Salas *et al.* 2003; de Salas *et al.* 2004a, 2004b; Flø Jørgensen *et al.* 2004).

In addition, several different cyst types were identified in the genus *Scrippsiella* (see Chapters 2 and 4). By cyst germination and molecular studies, another novel species (*Scrippsiella irregularis*) was identified with a cyst similar to the cysts of *Scrippsiella precaria*, but its theca and nucleotide sequence of ITS-rDNA was distinct (see Chapter 4). The present molecular study of all Calciodinellaceae, including fossil based and extant species, is in concordance with previous studies of this group (e.g. Montresor *et al.* 2003; Gottschling *et al.* 2005a). There are several cryptic species within the *Scrippsiella* group and their phylogenetic relationships are not clear (e.g. Chapter 4; Montresor *et al.* 2003; Gottschling *et al.* 2005a). Presently, the assessment of the phylogenetic value of some commonly used morphological characters of the motile cells such as morphology of the sulcal plates, cell shape and cyst ornamentation is not possible (Montresor *et al.* 2003). For example, this study found some other puzzling species such as *Scrippsiella* sp. 1 and *Scrippsiella* sp. 2 that were morphologically and genetically different from other members of the genus. *Scrippsiella* sp. 1 resembles *S. trochoidea* but genetically is allied to strains of *S. trochoidea* var. *aciculifera*. *Scrippsiella* sp. 2 is also possibly a new species of *Scrippsiella* (see Chapter 4). To define and introduce these new species in such a complicated group, further studies on the ecology, life cycle and mating system, crystallographic orientation of the cyst ornamentation and genetic variability are required.

Another finding of this research is the presence of *Chattonella* cf. *subsalsa* in the study area. There is almost no comparable information on this group from adjacent waters (i.e. the Arabian Sea, the Gulf of Oman and the Persian Gulf) to compare with this Iranian isolate. The morphological and molecular analyses presented show that this strain has a close relationship to *Chattonella subsalsa*, but is morphologically

and genetically distinct (Chapter 5) and is likely to be represent a new distinct species of *Chattonella.*

Harmful Algal blooms in the south Iranian coast cause a serious threat in the area for the marine ecosystem, fisheries and aquaculture. This study provides information on the cyst flora and presence of potentially harmful dinoflagellate and raphidophytes in an area that has potential for development of shellfish aquaculture. Furthermore, it also provides initiative(s) for future investigation in this field. While this study found few potentially toxic species, an increase in potentially toxic species in the Iran's south coasts may occur in the future due to growing international shipping and ballast exchange, or monsoon circulation that may introduce new species to the area.

In Summary, the results of this study are:

1. The cyst assemblage of Iranian sediment is dominated by *Scrippsiella* spp. and particularly has a high relative abundance of *Scrippsiella trochoidea* and with consistent occurrence of the species in all sites. Eight cyst types were identified in this group and one new species was described by cyst germination and molecular study.

2. *Protoperidinium* appears to be the most diverse genus of the cyst flora. *Protoperidinium oblongum* and round brown cyst of *Protoperidinium* sp.7 were found in higher concentration in this genus. Occurrence of the most species was not consistent among sites and more restricted to the bay environment of Chabahar. Some species in this group have not previously been described.

3. Only *Gymnodinium* sp. and *Polykrikos kofoidii* were identified among gymnodinioids. Microreticulate cyst of *Gymnodinium* sp. was identified as a novel species (*Gymnodinium trapeziforme*) by germination and molecular data. High cyst concentration was also found in the study areas, particularly in Bahoo-Kalat estuary.

4. In the study area, two cyst types of potentially toxic species *Alexandrium* cf. *tamarense* and *Lingulodinium polyedrum* were identified. Cysts of *Pyrodinium bahamense* (a typical warm water toxic species) was not found in this study.

5. Among potentially harmful raphidophytes only *Chattonella* cf. *subsalsa* was found in Pasabandar coastal region.

Finally, this study revealed that we are dealing with several unique and novel species in this tropical area, that can provide a background for further research in remaining areas of Iran's south coasts not covered in this study.

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Appendices

Appendix 1

GSe Medium (Modification of G.P. Medium)

1. Seawater

Filtered seawater autoclaved in 1liter Teflon bottles.

2. Stock Solution

1. KNO_3		100 gL^{-1} H_2O
2. K_2HPO_4		34.8 gL^{-1} H_2O
3. Vitamins		
	Biotin	0.2 mg 100 mL^{-1} H_2O
	B_{12}	0.1 mg 100 mL^{-1} H_2O
	Thiamin HCL	100.0 mg100 mL^{-1} H_2O
4. PII Metal Mix		
	Na_2EDTA	6.0 gL^{-1} H_2O
	$FeCl_3.6H_2O$	0.29 gL^{-1} H_2O
	H_3BO_3	6.85 gL^{-1} H_2O
	$MnCl_2.4H_2O$	0.86 gL^{-1} H_2O
	$ZnCl_2$	0.06 gL^{-1} H_2O
	$CoCl_2.6H_2O$	0.026 gL^{-1} H_2O
5. Selenium	H_2SeO_3	1.29 mgL^{-1} H_2O
6. Extracted soli		

3. Nutrient Solution (excluding soil extract)

To prepare GSe medium, solution of nutrients is made up for 100 ml mix:

Nitrate stock	20ml
Phosphate stock	10ml
Vitamin stock	10ml
PII Metal stock	50ml
Selenium stock	10ml

Make up to 200ml with distilled water

Nutrient solution was autoclaved in Schott bottles, then filter sterilised using 0.22 μm filter.

4. To Prepare Final GSe Medium

In a sterile 1 litre Teflon bottle the following concentration were added.

1000 ml sterile filtered Seawater (1)

20ml nutrient solution (3)
5ml extracted soil

Modification of GSe Medium

GSe medium without extracted soil was used in culture flasks. GSe^{-N-P} was also used in this study that contains the same amount of nutrients but without nitrate and phosphate.

Table 1: Dinoflagellate cyst counted in each site

Cyst Species	Stations								
	Bahoo-Kalat Estuary			Pasabandar Coast			Chabahar Bay		
	1	2	3	4	5	6	7	8	9
Gonyaulax cf. *digitale*	0	0	0	0	0	2	2	2	3
Spiniferites mirabilis	0	1	0	0	3	2	1	5	3
Spiniferites cf. *mirabilis*	0	0	0	0	0	0	0	0	1
Gonyaulax membranaceus	0	0	1	27	7	2	2	7	6
Spiniferites ramous	0	0	0	0	0	0	1	0	0
Gonyaulax baltica	0	0	0	0	3	0	3	2	5
Lingulodinium polyedrum	0	0	0	0	5	2	3	13	15
Gonyaulax sp. 1	0	0	1	27	0	2	0	2	3
Gonyaulax sp. 2	0	0	0	0	0	0	1	0	0
Gonyaulax sp. 3	0	0	0	0	0	0	1	0	0
Alexandrium cf. *tamarense*	0	0	0	33	10	3	0	0	0
Alexandrium cf. sp. 1	0	0	0	0	0	2	0	0	0
Pyrophacus steinii	0	0	0	0	0	0	1	0	1
Scrippsiella spp.	48	59	221	3520	1300	1829	141	169	196
Scrippsiella sp. 2	7	3	15	667	83	62	0	0	0
Protoperidinium avellana	0	0	0	0	3	2	0	0	0
P. cf. *denticulatum*	0	0	0	0	7	0	0	0	0
Protoperidinium claudicans	0	0	0	0	0	0	1	0	0
Protoperidinium conicum	0	0	0	0	0	0	2	1	0
P. cf. *thorianum*	0	0	0	0	0	0	1	0	0
P. cf. *punctulatum*	1	0	0	0	0	0	0	0	0
Protoperidinium minutum	0	0	0	0	3	5	0	0	0
Protoperidinium leonis	1	0	2	0	7	11	7	3	2
Protoperidinium oblongum	0	2	2	0	7	25	35	65	35
Protoperidinium pentagonum	1	0	0	0	0	0	7	16	7
Protoperidinium sp.1	0	0	0	0	0	0	1	0	0
Protoperidinium subinerme	0	0	0	0	0	0	6	4	2
P. cf. *conicoides*	0	0	0	0	0	0	1	0	0
Protoperidinium sp. 2	0	0	0	0	13	0	3	0	0
Protoperidinium sp. 3	0	0	0	0	0	0	0	1	0
Protoperidinium sp. 4	0	0	0	0	0	0	0	0	3
Protoperidinium sp. 5	0	0	0	0	0	0	1	0	0
Protoperidinium sp. 6	0	0	0	0	0	0	0	1	0
Protoperidinium sp. 7	1	2	4	0	20	58	77	114	111
Protoperidinium sp. 8	0	0	0	0	0	0	0	1	1
Diplopsalis sp.	0	0	0	0	0	0	1	0	0
Zygabikodinium leticulatum	0	0	0	0	0	4	0	2	0
Gymnodinium trapeziforme	4	6	9	280	231	29	0	0	0
Polykrikos kofoidii	0	0	0	0	3	0	0	0	0
Unknown	9	8	5	47	71	32	3	16	16

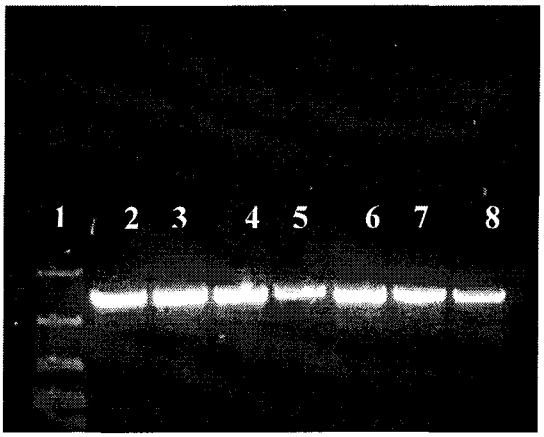
Table 2: List of dinoflagellate and raphidophyte strains used in this study (excluding 20 *Scrippsiella* strains isolated from mixed-incubated sediments)

Species	Strain Code	Location	Date of Sampling	Date of Isolation
Gymnodinium trapeziforme	GYPC102 GYPC104	Pasabandar Pasabandar	17.03.2004	2.09.2004 2.09.2004
Scrippsiella irregularis	SCBC17 SCBC19	Bahoo-Kalat Bahoo-Kalat	16.03.2004 16.03.2004	22.06.2004 22.06.2004
Scrippsiella sp. 1	SCPC21 SCPC23	Pasabandar Pasabandar	17.03.2004	7.04.2004 7.04.2004
Scrippsiella sp. 2	SCPC116	Pasabandar	17.03.2004	6.09.2004
Scrippsiella trochoidea	SCBC18 SCPC36 SCPC39 SCPC51 SCPC73	Bahoo-Kalat Pasabandar Pasabandar Pasabandar Pasabandar	16.03.2004 17.03.2004 17.03.2004 17.03.2004 17.03.2004	22.06.2004 8.04.2004 8.04.2004 8.04.2004 19.08.2004
Chattonella cf. *subsalsa*	CHPI36	Pasabandar	17.03.2004	6.09.2004

Strain code: (first two letter refer to genus e.g:GY=Gymnodinium), third letter refer to locations P=Pasabnadar coast or B=Bahoo-Kalat estuary, C=germinated from single cyst or I= isolated from incubated mixed sediment, number refer to strain number e.g.102.

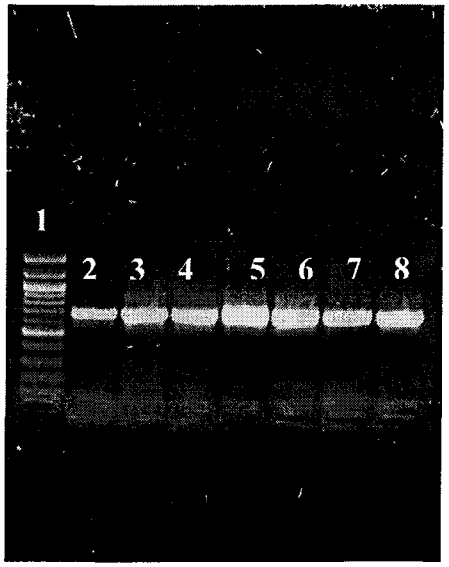
Appendix 2

Fig. 1. PCR product of *Scrippsiella* species in 1% agarose/TBE gel stained with Ethidium bromide, using D1R-F and 1483-R primers to amplify partial LSU (≈1400bp).



Lane 1= Ladder 50-2000 bp size standard
Lane 2= Strain SCBC17
Lane 3= Strain SCBC18
Lane 4= Strain SCBC19
Lane 5= Strain SCPC21
Lane 6= Strain SCPC23
Lane 7= Strain SCPC36
Lane 8= Strain SCPC39

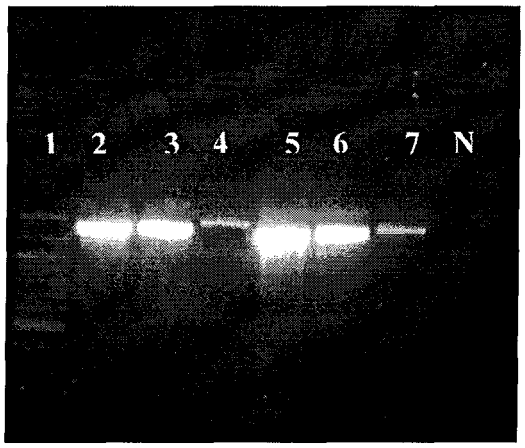
Fig. 2. PCR product of *Scrippsiella* and *Gymnodinium* strains using ITS-A and ITS-B primers



Lane 1= 100 bp DNA Ladder
Lane 2= Strain GYPC104
Lane 3= Strain SCPC51
Lane 4= Strain SCPI25
Lane 5= Strain SCPI11
Lane 6= Strain SCPI4
Lane 7= Strain SCPI16
Lane 8= Strain SCPI8.

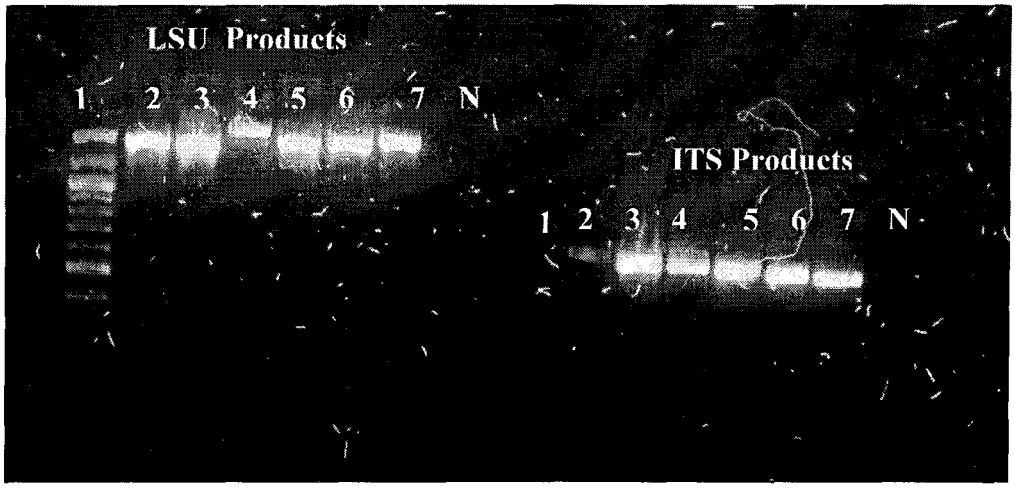
Note, Lanes 4-8 are *Scrippsiella* strains established from individual cell isolated from mixed-incubated sediments (see Chapter 4).

Fig. 3. PCR product of different species using D1R-F and 1483-R primers.



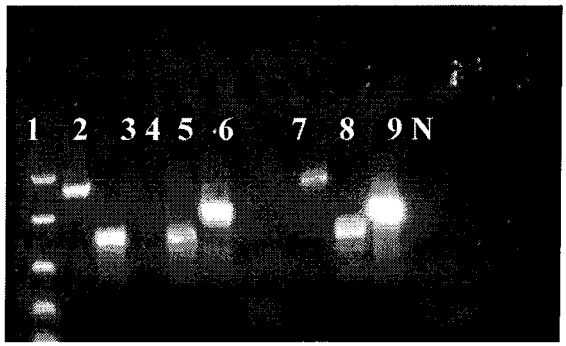
Lane 1= 100 bp DNA Ladder
Lane 2= Strain SCPC51
Lane 3= Strain CHPI36
Lane 4= Strain SCPC19
Lane 5= Strain SCPC23
Lane 6= Strain SCPI4
Lane 7= Strain GYPC104
N= Negative control

Fig. 4. PCR products of different species using D1R-F and 1483-R primers to amplify D1-D3 domains of LSU-rDNA (top bands) and ITS-A /ITS-B to amplify ITS-rDNA regions (bottom bands)(≈700bp). 1% agarose gel was pre-stained with Ethidium bromide.



Lane 1= 100 bp DNA Ladder
Lane 2= Strain SCPC73
Lane 3= Strain SCBC17
Lane 5= Strain SCPC116
N= Negative control
Lane 7= Strain GYPC102
Lane 8= negative control
Lane 4= Strain CHPI36
Lane 6= Strain SCPC21

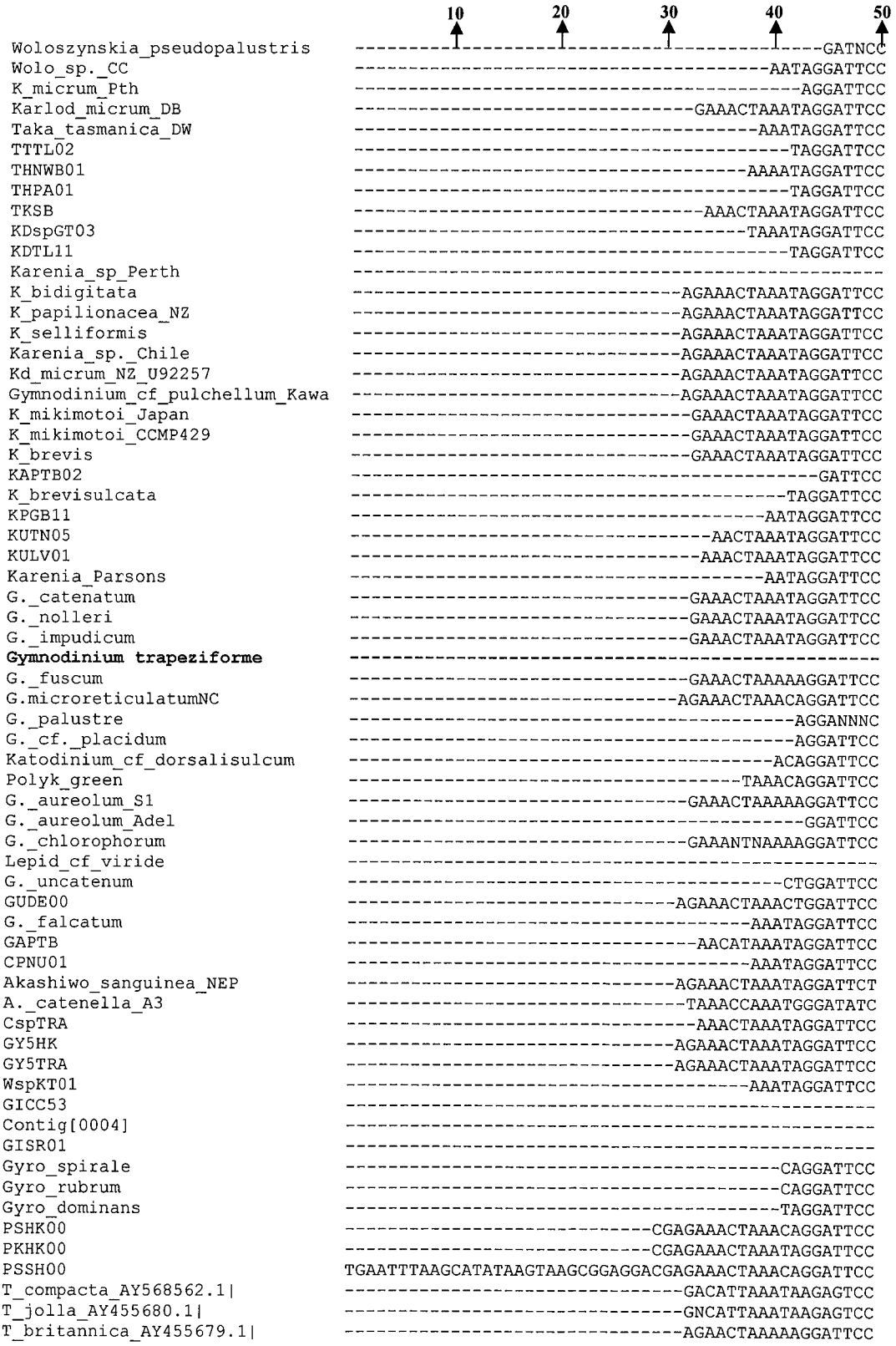
Fig. 5. PCR product of *Gymnodinium trapeziforme* and *Chattonella* cf. *subsalsa,* using three different reverse primers.



Lane 1= 2000 bp DNA LadderI
Lane 2= Strain GYPC102 (1483-R and D1R-F primers)
Lane 3= Strain GYPC102 (D2C-R and D1R-F primers)
Lane 4= Strain CHPI36 (1483-R and D1R-F primers)
Lane 5= Strain CHPI36 (D2C-R and D1R-F primers)
Lane 6= Strain CHPI36 (D3B*-R and D1R-F primer)
Lane 7= Strain GYPC104 (1483-R and D1R-F primers)
Lane 8= Strain GYPC104 (D2C-R and D1R-F primers)
Lane 9= Strain GYPC102 (D3B*-R and D1R-F primer)
Lane 10= Negative control using MiliQ-water

Appendix 3

Figure 1. Sequence alignment of partial LSU-rDNA region (domains D1-D3) in Gymnodinioid dinoflagellate species



```
                                  51                                                  100
                                  ↑                                                   ↑
Woloszynskia_pseudopalustris      CTTAGTAATGGCGAACGAACAGGGATGAGCTCGGCTTGGAAACTGGGGCC
Wolo_sp._CC                       CTTAGTAATGGCGAACGAACAGGGATGAGCTCGGCTTGGAAACTGGGGCC
K_micrum_Pth                      CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCTTGGAAATTGGGCCC
Karlod_micrum_DB                  CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCTTGGAAATTGGGCCC
Taka_tasmanica_DW                 CTCAGTAATGGCGAATGAACAGGGATCAGCTCAGCATGGAAATTGGGGCC
TTTL02                            CTCAGTAATGGCGAATGAACAGGGATCAGCTCAGCATGGAAATTGGGGCC
THNWB01                           CTCAGTAATGGCGAATGAACAGGGATCAGCTCAGCATGGAAATTGGGGCC
THPA01                            CTCAGTAATGGCGAATGAACAGGGATCAGCTCAGCATGGAAATTGGGGCC
TKSB                              CTCAGTAATGGCGAATGAACAGGGATCAGCTCAGCATGGAAATTGGGGCC
KDspGT03                          CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
KDTL11                            CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
Karenia_sp_Perth                  ----------------------------------------AATTGGGGCC
K_bidigitata                      CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
K_papilionacea_NZ                 CTTAGTAATGGCGAATGAACAGGGATAAKCTCAGCATGGAAATTGGGGCC
K_selliformis                     CTTAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
Karenia_sp._Chile                 CTTAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
Kd_micrum_NZ_U92257               CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCTTGGAAATTGGGCCC
Gymnodinium_cf_pulchellum_Kawa    CTCAGTAATGGCGAATGAACAGGGATCAGCTCAGCATGGAAATTGGGGCC
K_mikimotoi_Japan                 CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
K_mikimotoi_CCMP429               CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
K_brevis                          CTCAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
KAPTB02                           CTTAGTAATGGCGAATGAACAGGGACAAGCTCAGCATGGAAATTGGGGCC
K_brevisulcata                    CTTAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
KPGB11                            CTTAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
KUTN05                            CTTAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
KULV01                            CTTAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
Karenia_Parsons                   CTTAGTAATGGCGAATGAACAGGGATAAGCTCAGCATGGAAATTGGGGCC
G._catenatum                      CTTAGTAATGGCGAATGAACAGGGATGAGCTCAACATGGAAATTTTGGCT
G._nolleri                        CTTAGTAATGGCGAATGAACAGGGATGAGCTCAACATGGAAATTTTGGCT
G._impudicum                      CTCATTAATGGCGAATGAACAGGGATCAGCTCAACATGGAAATTGGGGCT
Gymnodinium trapeziforme          ----------------------------------------AGWGGCT
G._fuscum                         CTCAGTAATGGCGAATGAACAGGGAGCAGCTCAACATGGAAATCGTGGCC
G.microreticulatumNC              CTCAGTAATGGCGAATGAACAGGGATGAGCTCAACATGGAAATTGTGGCA
G._palustre                       CTCAGTAATGGCGAATGAACAGGGATGAGCTCACCATGGAAATTGTAGCT
G._cf._placidum                   CCCAGTAATGGCGAATGAACAGGGATGAGCTCACCATGGAAATTGTAGCT
Katodinium_cf_dorsalisulcum       CTTAGTAATGGCGAATGAACAGGGATGAGCTCAACATGGAAATTGAGGCT
Polyk_green                       CTTAGTAATGGCGAATGAATAGGGATGAGCTCAACGTGGGAATTGTGGCT
G._aureolum_S1                    CTTAGTAATGGCGAATGAACAGGGATGAGCTCAATATGGAAATTGTGGCT
G._aureolum_Adel                  CTTAGTAATGGCGAATGAACAGGGATGAGCTCAATATGGAAATTGTGGCT
G._chlorophorum                   CTTAGTAATGGCGAATGAACAGGGATGAGCTCAACTTGATAATTGTGGCT
Lepid_cf_viride                   --------TGGCGAATGAACAGGGATGAGCTCAACTTGATAATTGTGGCT
G._uncatenum                      CTAAGTAATGGCGAATGAACAGGGATGAGCTCATTGTTAAAATCAGGGTC
GUDE00                            CTAAGTAATGGCGAATGAACAGGGATGAGCTCATTGTTAAAATCAGGGTC
G._falcatum                       TTAAGTAATGGCGAATGAACAAGGATGAGCTCAGTATGAAAATCGGGGCC
GAPTB                             CTCAGTAATGGCGAATGAACAGGGAAGAGCTCAGAATGAAAATCGGGGCT
CPNU01                            TTAAGTAATGGCGAATGAACAAGGATGAGCTCAGAATGAAAATCGGGGCC
Akashiwo_sanguinea_NEP            CTTAGTAATGGCGAATGAACTGGGATGAGCTCACCATGGGAATCGGGGTC
A._catenella_A3                   TTTAGTAATTGCGAATGAACAAGGATATGCTTAGCTTGACAAATGGAGCT
CspTRA                            CTTAGTAATGGCGAATGAACAGGGATAAGCTCAACTTGGAAATTGTGGCC
GY5HK                             CTCAGTAATGGCGAATGAACAGGGAGAAGCTCAACATGGAAATTGGGGCC
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WspKT01                           CCCAGTAATGGCGAATGAACAGGGATGAGCTCGGCTTGGAAACTGGGGCC
GICC53                            AAGCGGAGGATAAGAAACTAAACAGGATTCCCTTAGTAATGGCGAATGAA
Contig[0004]                      ----------------TATACTCATGTATGACGAACGATTTGCACGTCAG
GISR01                            --------------------------------------------------
Gyro_spirale                      CTCAGTAATGGCGAATGACCAGGGATAAGCTCAGCATTGAAATCTGGGCC
Gyro_rubrum                       CTCAGTAATGGCGAATGAACAGGGATGAGCTCAGCATTGAAATTTGGGCC
Gyro_dominans                     CTTAGTAATGGCGAATGAACAGGGATGAGCTCAGCATTAAAATCTGGGCC
PSHK00                            CACAGTAATGGCGAATGAACGTGGAGGAGCTCAACATGAGAATTGTGGCC
PKHK00                            CACAGTAATGGCGAATGAACGTGGAGGAGCTCAGCNTGAGAATTGTGGCC
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T_compacta_AY568562.1|            CTTAGTAGTGGCGAACGAACAGGGATTGGCTCACCCTAAAAATCAGGGCC
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T_britannica_AY455679.1|          CTCAGTAATGGCGAATGAACAGGGATAGGCTCAATGTGGAATCCAGGGCC
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                                101                                 150
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Woloszynskia_pseudopalustris    TT-GGCCTTGGATTGTAGCCTCTAGACA-TAGCA-CCAACG-GAGGCGCA
Wolo_sp._CC                     TC-GGCCTTGGATTGTAGCCTCTAGACA-TAGCA-CCAACG-GAGGCGCA
K_micrum_Pth                    TCTGGGCTTGAATTGTAGTCTTTCGATGGCATTG-CCAGCG-GAGGCGCA
Karlod_micrum_DB                TCTGGGCTTGAATTGTAGTCTTTCGATGGCATTG-CCAGCG-GAGGCGCA
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K_papilionacea_NZ               TCTGGCCTTGAATTGTAGTCTTGAGATG-TGCTG-CCAACG-GAGGCGCA
K_selliformis                   TCTGGCCTTGAATTGTAGTCTTGAGATG-TGCTA-CCAACG-GAGGCGCA
Karenia_sp._Chile               TCTGGCCTTGAATTGTAGTCTTGAGATG-TGCTA-CCAACG-GAGGCGCA
Kd_micrum_NZ_U92257             TCTGGGCTTGAATTGTAGTCTTTCGATGGCATTG-CCAGCG-GAGGCGCA
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K_mikimotoi_CCMP429             CTCGGCCTTGAATTGTAGTCTTGAGATG-TGTTA-CCAACG-GAGGCGCA
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G._catenatum                    CCTGGCCACGAATTGTAATCTCTCGACG-CGCTG-CCAGCA-GGGGCGTA
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G._impudicum                    TCTGGCTTTGAATTGTAATCTCAAGATG-TATTG-CCAACA-GGGGCGCA
Gymnodinium trapeziforme        TCTCGCCATGAAWTGTAATCTCTCGATG-CATTG-CCAATG-GAGGCGCA
G._fuscum                       TGTGGCCATGAATTGTAATTTCAAGACG-CATTG-CCAACG-GTGGCGCA
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G._aureolum_Adel                TCTGGCTTTGAATTGTAATCTCAAGATG-CATTG-CTAACT-GGGGCGTA
G._chlorophorum                 TCTGGCTGTGAATTGTAATCTCAAGATG-TAGTG-CCAAGG-AGGGCGTA
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CPNU01                          TATGGCTTTGAATTGTAGTCTTGAGATG-TGTTG-CCAGCG-GAGGCGCA
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GISR01                          -------------------------ATG-TATTG-CCAACA-GGGGCGCA
Gyro_spirale                    CTCGGCCTTGAATTGTAATCTCGAGATG-TGCAA-CCAACG-GAGGCGCA
Gyro_rubrum                     CCCGGCCTTGAATTGTAATTTCGAGATG-TGCAA-CCAATG-GAGGCGCA
Gyro_dominans                   CCCGGCCTTGAATTGTAATCTCGAGAAG-TGCAA-CCAACG-GCGGCGCA
PSHK00                          TTCGGCCTTGAATTGTAATCTCAAGAT-GTACTG-CCAACGG-AGGCGCA
PKHK00                          TTCGGCCTTGAATTGTAATCTGAAGAT-GTGTTGGCCAACGG-AGTCGCA
PSSH00                          TTCGGCCTTGAATTGTAATCTCAAGAT-GTACTG-CCAACGG-AGGCGCA
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T_jolla_AY455680.1|             TCAGGTCATGAATTGTAGTCTCTAGATG-TGTTG-CCATCA-GGGGTGCT
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K_papilionacea_NZ               GATGTAAGCCTCTTG-GAAAAGAGCATCAGGGAGGGTGAGAGTCCCGTAT
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A._catenella_A3                 GGTGCCAGCCTATTG-AAATAAAGCGTCAATGAGGGTGAGAATCCTGTTT
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GICC53                          ATCTCAAGATG-TACTG-CTAGCGG-GGGCGCGGGTGAAAGCCTCTTG-G
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Gyro_rubrum                     GGTGCAAGCCGCTTG-GAAAAGCGCACCACCGAGGGTGAGAGTCCCGTTC
Gyro_dominans                   GGTGTAAGCCTCTTG-GAAAAGAGCACCAATGAGGGTGAGAGTCCCGTTT
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Wolo_sp._CC                       TTCATCTGCAGTCCACCGTGCACGGTGCATGTTCTCAGAGTCGCGTTCCT
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Karlod_micrum_DB                  GTCATCTGCAGTCCTCTGTTTACGGTGTGCTTTCTAAGAGTCACGTTCCT
Taka_tasmanica_DW                 GTCATCTGCAGTCCCCCGTGCACGGCTTGCTTTCTAAGAGTCACGTTCCT
TTTL02                            GTCATCTGCAGTCCCC-GTGCACGGCTTGCTTTCTAAGAGTCACGTTCCT
THNWB01                           GTCATCTGCAGTCCCCCGTGCACGGCTTGCTTTCTAAGAGTCACGTTCCT
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TKSB                              GTCATCTGCAGTCCCCCGTTCACGGTGTGCTTTCTAAGAGTCACGTTCCT
KDspGT03                          GTCATCTGCAGTCCCCCATAAACGGTGTGCTTTCTAAGAGTCACGTTCCT
KDTL11                            GTCATCTGCAGTCCCCCATAAACGGTGTGCTTTCTAAGAGTCACGTTCCT
Karenia_sp_Perth                  GTCATCTGCAGTCCCCCGTGCACGGCGTCGGTTCTAAGAGTCACGTTCCT
K_bidigitata                      GTCATCTGCAGTTCTCCGTGCACGGTGCATTTTCTAAGAGTCACGTTCCT
K_papilionacea_NZ                 GTCATCTGCAGATCTCTGTGCACGGTGCGCTTTCTAAGAGTCACGTTCCT
K_selliformis                     GTCATCTGCAGTTCTCTGTGCACGGTACATTTTCCAAGAGTCACGTTCCT
Karenia_sp._Chile                 GTCATCTGCAGTTCTCTGTGCACGGTACATTTTCTAAGAGTCACGTTCCT
Kd_micrum_NZ_U92257               GTCATCTGCAGTCCTCTGTTTACGGTGTGCTTTCTAAGAGTCACGTTCCT
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Lepid_cf_viride                   GTCACCTGCAGTCCCCCCTGTACGGCCTACCTTCTAAGAGTCACGTTCCT
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GUDE00                            ATCACCTGTGGCCCCCTGTGCTCGGCGCGCTTTCTAAGAGTCACGCTCCT
G._falcatum                       GCCATCTGCAGCCCCCCGTGTACGGCGCATGTTCTAAGAGTCACGTTCCT
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Gyro_spirale                      GTCATTTGCAGTCCTCCGTGTACGGTGTACTTTCTAAGAGTCACGTTCCT
Gyro_rubrum                       GTCACTTGCAGTCCTCCGTGNACGGTGTGCTTTCTAAGAGTCACGTTCCT
Gyro_dominans                     GTCATCTGCAGTCCTCTGTGTACGGTGTGCTTTCTAAGAGTCACGTTCCT
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Wolo_sp._CC                       GGCTTGAGACCGATAGCAAACAAGTACCATGAGGGAAAGATGAAAAGGAC
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Karlod_micrum_DB                  GGTTCGAGACCGATAGCAAACAAGTACCATGAGGGAAAGGTGAAAAGGAC
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Polyk_green                       GGTTCGAGACCAATAGCAAACAAGTACCATGAGGGAAAGATGAAAAGGAC
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G._chlorophorum                   GGTTCGAAACCGATAGCAAACAAGTACCATGAGGGAAAGATGAAAAGGAC
Lepid_cf_viride                   GGTTCGAAACCGATAGCAAACAAGTACCATGAGGGAAAGATGAAAAGGAC
G._uncatenum                      GGTTCGAGACCGATAGCAAACAAGTACCATGAGGGAAAGGTGAAAAGGAC
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G._falcatum                       GGTTCGAGACCGATAGCAAACAAGTACCATGAGGGAAAGGTGAAAAGGAC
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Akashiwo_sanguinea_NEP            GGTCCGAGACCGATAGCAAACAAGTACCATGAGGGAAAGGTGAAAAGGAC
A._catenella_A3                   CAATTGAGACTGATAGCACACAAGTACCATGAGGGAAATATGAAAAGGAC
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GICC53                            ATTGGGTGGTAAATTTCATCCCAAGCTAAATATGGGTTCGAAACCGATAG
Contig[0004]                      TGTTAGACTCCTTGGTCCGTGTTTCAAGACGGGTCGAATGGAACCATTTA
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Gyro_spirale                      GGTTCGAGACCGATAGCAACCAAGTCCCATGAGGGAAAGATGAAAAGGCC
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Gyro_dominans                     GGTTTGAGACCGATAGCAAACAAGTACCATGAGGGAAAGATGAAAAGGAC
PSHK00                            GGTTCGAGACCGATAGCAAACAAGTACCATGAGGGAAAGATGAAAAGGAC
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Wolo_sp._CC                       TTTGAAAAGAGAGTTAAAAGTGCTTGAAATTGCTGAAAGGGAAGCGAATG
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Karlod_micrum_DB                  TTTGAAAAGAGAGTTAAAAGTGCCTGAAATTGCTGAAAGGGAAGCGAATG
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K_bidigitata                      TTTGAAAAGAGAGTTAAAAGTGCCTGAAATTGCTGAAAGGGAAGCGAATG
K_papilionacea_NZ                 TTTGAAAAGAGAGTTAAAAGTGCCTGAAATTGCTGAAAGGGAAGCGAATG
K_selliformis                     TTTGAAAAGAGAGTTAAAAGTGCCTGAAATTGCTGAAAGGGAAGCGAATG
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Kd_micrum_NZ_U92257               TTTGAAAAGAGAGTTAAAAGTGCCTGAAATTGCTGAAAGGGAAGCGAATG
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G._nolleri                        TTTGAAAAGAGAGTTAAAAGTGCCTGAACTTGCTGAAAGGAAAGCGGATG
G._impudicum                      TTTGAAAAGAGAGTTAAAAGTGCCTGAACTTGCTGAAAGGAAAGCGGATG
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G._fuscum                         TTTGAAAAGAGAGTTAAAAGTGCTTGAACTTGCTGGAAGGAAAGCGGATG
G.microreticulatumNC              TTTGAAAAGAGAGTTAAAAGTGCCTGAACTTGCTGAAACGAAAGCGGATG
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Polyk_green                       TTTGGAAAGAGAGTTAAACGTGCCTGAACTTGTTGAAAGGGAAGCGGATG
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Lepid_cf_viride                   TTTGAAAAGAGAGTTAAAAGTGCCTGAACTTGCTGAAAGGAAAGCGGATG
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WspKT01                           TTTGAAAAGAGAGTTAAAAGTGCTTGAAATTGCTGAAAGGGAAGCGAATG
GICC53                            CAAACAAGTACCATGAGGGAAAGATGAAAAGGACTTTGAAAAGAGAGTTA
Contig[0004]                      GTCACCATG-GTTGTGTGTGAAAGGTGCGCGCGCGCACTGCCGCAGCCTT
GISR01                            TTTGAAAAGAGAGTTAAAAGTGCCTGAACTTGCTGAAAGGAAAGCGGATG
Gyro_spirale                      TTTGGAAAGAGAGTTAAAAGTGCCTGAAATCGCTGAAAGGGAAGCGAATG
Gyro_rubrum                       TTTTGAAAGAGAGTTAAAAGTGCCTGAAATTGCTGAAAGGGAAGCGAATG
Gyro_dominans                     TTTGGAAAGAGAGTTAAAAGTGCCTGAAATCGCTGAAAGGGAAGCGAATG
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Wolo_sp._CC                     GAACCAGTCCT-CTGGCTGAG-ATTGCTGCAT-----------------
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Karlod_micrum_DB                GAACCAGTCTA-CTTGGTGAG-ATTGTTGTGCGCTATT-GTGATTGCTTT
Taka_tasmanica_DW               GAACCAGTTTTTCTTGGCGAG-ATTGTTGCGTGCTAAT-GTGATGGCTTG
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K_papilionacea_NZ               GAACCAGTTGTTCTTGGCTAG-ATTGGTGTTGTCTAAT-GTGATGGCTTG
K_selliformis                   GAACCAGTTGTTCTTGGCGAGTATTGGTGTTGTCTAAA-GTGAGGGCTTG
Karenia_sp._Chile               GAACCAGTTGTTCTTGGCGAGTATTGGTGTTGTCTAAA-GTGAGGGCTTG
Kd_micrum_NZ_U92257             GAACCAGTCTT-CTTGGTGAG-ATTGTTGTGCGCTATT-GTGATTGCTTT
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Karenia_Parsons                 GAACCAGTTGTTCTTGGCGAGTATTGGTGTTGTCTAAA-GTGATGGCTTG
G._catenatum                    GAACCAGTTTTGCTTGGTGAG-ATTGTCGCACGCAGCA-ATGATCACCTT
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G.microreticulatumNC            GAACCAGTCT-GCTTGGTGAG-ATTGTCGCGTGCTGCA-TTGATTGTCTG
G._palustre                     GAACCACCATTGCGTGGCGAG-ATTGTTGTGCGCCTCA-GAGATTGCCTG
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Katodinium_cf_dorsalisulcum     GAACCAGTGTTGCTTGGTAAG-ATTGTTGCGCGCTATG-GTGATT-CTTG
Polyk_green                     GAACCAGTGCT--TTGGTGAG-ACTGCTGCATTCCATA-TTGATTACCCG
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Lepid_cf_viride                 GAACCAGTGTTGCTCGGTGAG-ATTGTTGCGTGCTATG-GTGATGGCCTG
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Akashiwo_sanguinea_NEP          GAACCAGTTCTGCTGGGCGAG-ATTGTCGCAT-----------------
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WspKT01                         GAACCAGTCA-TCTGGCTGAG-ATTGTTGCAT-----------------
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GISR01                          GAACCAGTGTTGCTTAGTGAG-ATTGTTGCGCGCTAAG-GTGATGGCCTG
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Gyro_rubrum                     GAACCAGTGTTTCTTGGCGAG-ATTGTTACACGCTAAG--TGGATAGTCG
Gyro_dominans                   GAACCAGTGTTTCTTGCAGAG-CTTGGCGAGAGTAATC--TGGATGGTGC
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PKHK00                          GAACCAGCTTTGCTTGGCAAG-ATTGTTGTCGACCCGT-CTCGTGTGCGA
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Wolo_sp._CC                       ----------------------GCTTT-------------------TGT
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Karlod_micrum_DB                  CT-GCT------TCAACGCAAGTGTTGCACTGGGTTTTGAG-CATGACGC
Taka_tasmanica_DW                 TT-GCT------TCAACGCAAGTGTTGCAGCGGGTTTTGAT-CTGGACGT
TTTL02                            TT-GCT------TCAACGCAAGTGTTGCAGCGGGTTTTGAT-CTGGACGT
THNWB01                           TT-GCT------TCAACGCAAGTGTTGCAGCGGGTTTTGAT-CTGGACGT
THPA01                            TT-GCT------TCAACGCAAGTGTTGCAGCGGGTTTTGAT-CTGGACGT
TKSB                              CT-GCT------TCAACGCAAGTGTTGCAGTGGGTTTTGAT-CTGGACGT
KDspGT03                          CT-GCT------TCAACGCAAGTGTCGCAGTGGGTTTTGAG-CATGACGT
KDTL11                            CT-GCT------TCAACGCAAGTGTCGCAGTGGGTTTTGAG-CATGACGT
Karenia_sp_Perth                  CT-GCT------CCAACGCAAGTGTTGCAGTGAGTTTTGAT-CTGGACGT
K_bidigitata                      CC-ACT------TCAACGCAAGTGTGGTGGCATGTTTTGAA-CTGGATGC
K_papilionacea_NZ                 CC-ACT------TCAACGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
K_selliformis                     CC-ACT------TCAGCGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
Karenia_sp._Chile                 CC-ACT------TCAGCGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
Kd_micrum_NZ_U92257               CT-GCT------TCAACGCAAGTGTTGCAGTGGGTTTTGAG-CATGACGC
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K_mikimotoi_Japan                 CC-ACT------TCAACGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
K_mikimotoi_CCMP429               CC-ACT------TCAACGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
K_brevis                          CC-ACT------TCAACGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
KAPTB02                           CC-ACT------TCAACGCAAGTGTGGTGGCATGTTTTGAA-CTGGATGC
K_brevisulcata                    CC-ACT------TCAGCGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
KPGB11                            CC-ACT------TCAACGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
KUTN05                            CC-ACC------TCAACGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
KULV01                            CC-ACC------TCAACGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
Karenia_Parsons                   CC-ACT------TCAACGCAAGTGTGGTGGCAGGTTTTGAT-CTGGATGC
G._catenatum                      CT-ATT------CCAGCGAAAGCGGGGCGGTGGGCTGTGAT-TTGTTCGG
G._nolleri                        CT-ATT------CCAGCGCAAGCGGGGTGGTGGGCTGTGAT-TTGTTCGG
G._impudicum                      CT-GCA------TCAACGCAAGCGTTGCTGTGGGTTTTGAT-CTGGTTGC
Gymnodinium trapeziforme          TTCGCA-------CAGCGCAAGCGTGCGGGTTTTCTGTGAT-GTGTTCGG
G._fuscum                         TT-GTG------TTAGCGCAAGCGGCGCATGGCGCTCTGAT-CTGAATGC
G.microreticulatumNC              CT-TGC------TCAGCGTAAGCGTGCTTGTAGTCTTTGAC-GTGTTCGC
G._palustre                       TC-GAG------CGAGCGCAAGCAGCTTGGTGGGTTCTGAT-CTGGCAAC
G._cf._placidum                   TC-GAG------CGAGCGCAAGCCGCTTGTTTGGTTTNGAT-CTGGCACA
Katodinium_cf_dorsalisulcum       CT-GCG------CCAGCGCAAGCGGTGCTGTGGGTTG-AAT-CTTGTTGC
Polyk_green                       CT-GTG------CCAGCGCAAGCAGTGCATTGGGTTCTGAT-GTGGACGA
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Lepid_cf_viride                   CT-GCA------TCAGCGCAAGCGTTGCTGTTGGTTTTGAT-CTTGATGC
G._uncatenum                      CT-CCG------GTAGTGCAAACACGGCTGCAGGCGTCGAT-CTGGTTGC
GUDE00                            CT-CCG------GTAGTGCAAACACGGCTGCAGGCGTCGAT-CTGGTTGC
G._falcatum                       CT-ATG------GTAACGCAAGTGCCCTAGCTTGCCACCAA-CTTGATGC
GAPTB                             CC-TGG------TCAACGCAAGTGTCCTGGCTGGCTCTCGA-CTTGATGC
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Akashiwo_sanguinea_NEP            ----------------GCCAAGGCCTTGT------------G-CCGGCCGC
A._catenella_A3                   -------------------ACAAGAGCT--T-----TGGGC-T---GTGG
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GY5HK                             TT-GCA------TCAGCGCAAGCGTTGCTTTGGGTCCTGAT-CTGGCTGC
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WspKT01                           ----------------------GCTCT------------------TGT
GICC53                            GGTAAG-------ATTGTCGCGTGCTAAGGTGAT-TCTCGC-TGCGT-CA
Contig[0004]                      CACAGT------CTGGCGGTGAGTTCCACAAGGCACGTTCG-CTCGCCCA
GISR01                            CT-GCA------TCAGCGCAAGCGTTGCTGTGGGTTTTGAT-CTGGTTGC
Gyro_spirale                      GTTGCT------TCAACGCAAGTGTTGCAGTTGGTTTTGAT-ATGGATGC
Gyro_rubrum                       GTTGCC------TCAACGCAAGTGTGGCTGCTGGCTTTGAT-GCGGACGT
Gyro_dominans                     TCTGAT------TCAGCGCAAGCGTTTCAGGGTTCTTTGA--AGGTGCAT
PSHK00                            AGGGGA-CAGGTTGAACTTGAAATTCCTTGTTCCTGGAAGTGC-ACGCGT
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Wolo_sp._CC                       GT---GTGGTGTTT-CTCAGCTTGTGAGTCAACCTTCGTTCATGCTTGAG
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Karlod_micrum_DB                  GC---ACTTTGTTT-CTCACCTCGTCTGTCAACGTCGGTTCAGATTTGAG
Taka_tasmanica_DW                 GC---GCGGTGCTT-CTTGCCTTGGGTGTCAACGTCGGTTCAGACTTGAG
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KDTL11                            GC---GCTTTGTTT-CTTGCCTCGTGTGTCAACGTCGGTTCGGACTTGAG
Karenia_sp_Perth                  GC---GCGGTGCTT-CTTGCCTTGTGTGTCAACGTCGGTGCCGACTTGAG
K_bidigitata                      G----ATACTGCTT-CTCGCCTTGCGTGTCAACGTCAGTTCATAATTGAG
K_papilionacea_NZ                 G----ATACTGGTTGCTTGCCTTGCGTGTCAACGTCAGTTCATAATTGAG
K_selliformis                     G----ATACTGCAT-CTCGCCTTGCATGTCAACGTCAGTTCATAATTGAG
Karenia_sp._Chile                 N----ATACTGCAT-CTCGCCTTGCATGTCAACGTCAGTTCATAATTGAG
Kd_micrum_NZ_U92257               GC---ACTTTGTTT-CTCACCTCGTGTGTCAACGTCGGTTCAGATTTGAG
Gymnodinium_cf_pulchellum_Kawa    GC---GCGGTGCTT-CTTGCCTTGGGTGTCAACGTCGGTTCAGACTTGAG
K_mikimotoi_Japan                 G----ATACTGCTT-CTCGCCTTGCATGTCAACGTCAGTTCATAATTGAG
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K_brevis                          G----ATACTGCTT-CTCGCCTTGCGTGTCAACGTCAGTTCATAATTGAG
KAPTB02                           G----ATACTGCTT-CTCGCCTTGCGTGTCAACGTCAGTTCTTAATTGAG
K_brevisulcata                    G----ATACTGAAT-CTCGCCTTGCATGTCAACGTCAATTCATAATTGAG
KPGB11                            G----ATACTGGTTGCTTGCCTTGCGTGTCAACGTCAGTTCATAATTGAG
KUTN05                            G----ATACTGCTT-CTCGCCTTGCATGTCAACGTCAGTTCACAATTGAG
KULV01                            G----ATACTGCTT-CTCGCCTTGCATGTCAACGTCAGTTCACAATTGAG
Karenia_Parsons                   G----ATACTGCTT-CTCGCCTTGCAAGTCAACGTCAGTTCATAATTGAG
G._catenatum                      -T---GTGATGTTT-CTTGCCTTGTGTGTCATCATCAGTTTGGAGGTGGG
G._nolleri                        GT---GTGATGTTT-CTTGCCTTGTGTGTCATCATCAGTTTGGCGGTGGG
G._impudicum                      GC---GTGATGCTT-CTTGCTTTGAACGTCATCTGGGGTTTGGAGGTGCT
Gymnodinium trapeziforme          GC---GTGATGTTT-CTTGCCTGGTGAGTCACTGGCAGTTTGCAGGTGGG
G._fuscum                         AC---GTGATGCTT-CTTACCTTGTGGGTCACCGTCAGCGTGGTGGCGAG
G.microreticulatumNC              GC---GTGATGTTT-CTTGCCTTGTCAGTCACTGTCAGTTGGCAGGCGAG
G._palustre                       GC---GCGATGCTT-CTTGCCTTGTGCGTCACTGACTGTTCGTCGGCGAG
G._cf._placidum                   CC---GCGATGCCT-CTTGCCTNGTGCGTCACTGGCTGTGCGTCGGCAAG
Katodinium_cf_dorsalisulcum       GC---GTGATGCTT-CTTGCCTTGTTCGTCACCGTCGGTTTGGGGGTGAG
Polyk_green                       AT---GCGATGTTT-CTTGCTTTGTGTGTCATCGCCAGTGTGATGATGAG
G._aureolum_S1                    GC---GCGATGCTT-CTTGCCTCGTGTGTCATCGTCAGTGTGCTGGTGAG
G._aureolum_Adel                  GC---GCGATGCTT-CTTGCCTCGTGTGTCATCGTCAGTGTGCTGGTGAG
G._chlorophorum                   GC---GTGATGCTT-CTTGCCTTGTGTGTCATCGTCGGTTCGGTGGTGAG
Lepid_cf_viride                   GC---GTGATGCTT-CTTGCCTTGTGTGTCATCGTCGGTTCGATGGTGAG
G._uncatenum                      GC---GTGGTCGCTTCTTGCTTTGGGTGTCAGCGTCAGCTCAACTCTGAG
GUDE00                            GC---GTGGTCGCTTCTTGCTTTGGGTGTCAGCGTCAGCTCAACTCTGAG
G._falcatum                       AT---GCGGT-GGTTCTTGCTTTGTGTGTCAGCTTTGGTGCAGACCTGAT
GAPTB                             AT---GCGGT-GGTTCTTGCCTTGTCTGTCAGCGCTGGCGTACACCTGAT
CPNU01                            AT---GCGGT-GGTTCTTGCTCTGTGTGTCAGCTTTGACGCAGACTTGAT
Akashiwo_sanguinea_NEP            AT---GCGGTGCTT-CTTGCCTTGTGTGTCAGCTGCGGTCCGAAGTTGGG
A._catenella_A3                   GT---GTAATGATT-CTTTCTTTGCATGCCAGTTTCTATTTGTACATCTG
CspTRA                            GC---GCGATGCTTC-TTGCCTTGTTCGTCATCGTTGGTTTGGGGGCGCG
GY5HK                             GC---GTGATGCTTC-TTGCCTTGTTTGTCACCGTCGGCTTCTTAGTGGG
GY5TRA                            GC---GTGATGCTTC-TTGCCTTGTTTGTCACCGTCGGCTTCTTAGTGGG
WspKT01                           GT---GTGATGCTT-CTCAGCTTGTTAGTCAACCATTGTTTAGGCTTGAG
GICC53                            -G---CGCAA---GCGGTGCTGTGGGTTG-GATCCTT---CCGCGC---G
Contig[0004]                      GG---GGCAA--GCTACCATGTCCCAAGAGTTGTCCTCACCTCCAAACCG
GISR01                            GC---GTGATGCTT-CTTGCTTTGAACGTCATCTGGGGTTTGGAGGTGCT
Gyro_spirale                      GC---GTGGTGCTT-CTCGCCTTGCTTGTCAACGTCGATTTGAAGTTGAG
Gyro_rubrum                       GT---GTGGTGCTT-CTTGCCTTGCCTGTCAACGTCAATTCGGGGTTGAG
Gyro_dominans                     TC---TTGCTGTTG-CTCTGCTTGCTTGTCAACGTCAATTTGGAGTTGGG
PSHK00                            GC--CGCGATGTTT-CTTGCCTTGCTTGTCACAAGCGGTTTGCGATTGAA
PKHK00                            GGGTCGCGATGTTT-CTTGCCTTGTGTGTCACAAACGGTTTGCGATTGAA
PSSH00                            GC--CGCGATGTTT-CTTGCCTTGCTTGTCACAAGCGGTTTGCGATTGAA
T_compacta_AY568562.1|            GC---ACAATGTTT-CTTGCCTTGCGTGTCATCTGCAGATCTGCACTGAG
T_jolla_AY455680.1|               GC---ACAATGTTT-CTCGCCCTGCGTGTCATCTGCAGATCTGCACTGAG
T_britannica_AY455679.1|          AT---GAGATGCTT-CTTTCCTTGGGTGTAATGTTCATTTCGTAGTTGAG
```

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                                  551                                                600
                                  ▲                                                  ▲
Woloszynskia_pseudopalustris      GATAGC--CTCAGGTTCACGAC-GCTTCCCCGC-GGGGTTGCTCCTGTGC
Wolo_sp._CC                       GATAGC--CTCAGGTTCACGAC-GCTTCCCCGC-GGGGTTGCTCCTGTGC
K_micrum_Pth                      GAAAAC--TCTAGGGACATGGTTAATCGCTCGC-GAGTGATTGAACGTGC
Karlod_micrum_DB                  GAAAAC--TCTAGGGACATGGTTAATTGCTCGC-GAGTGATTGAATGTGC
Taka_tasmanica_DW                 GAAAAC--TCCAGGGACATGGTTAGTCACTTCT-GGGTGACTGAACGTGC
TTTL02                            GAAAAC--TCCAGGGACATGGTTAGTCACTTCT-GGGTGACTGAACGTGC
THNWB01                           GAAAAC--TCCAGGGACATGGTAAGTCACTTCT-GGGTGACTGAAAGTGC
THPA01                            GAAAAC--TCCAGGGACATGGTAAGTCACTTCT-GGGTGACTGAAAGTGC
TKSB                              GAAAAC--TCCAGGGACATGGATAATCGCTCCT-GGGTGATTGAACGTGC
KDspGT03                          GAAAAC--TCCAGGGACATGGTAAATCGCTCGT-GGGTGATTGAATGTGC
KDTL11                            GAAAAC--TCCAGGGACATGGTAAATCGCTCGT-GGGTGATTGAATGTGC
Karenia_sp_Perth                  GATAAC--TCCAGGGACATGGTAAGTCGTCTGC-GG-TGACTGAATGTGC
K_bidigitata                      GAAAAC--TCTAGGGACATGGTAATTTGCTTCC-GGGTGACTGAATGTGC
K_papilionacea_NZ                 GAAAAC--TCTAGGGACATGGTAATTTGCTTCC-GGGTGACTGAATGTGC
K_selliformis                     GAAAAC--TCTAAGGACTTGGTAATTTGCTTCC-GGGTGACTGAATGTCT
Karenia_sp._Chile                 GAAAAC--TCTAAGGACTTGGTAATTTGCTTCC-GGGTGACTGAATGTCC
Kd_micrum_NZ_U92257               GAAAAC--TCTAGGGACATGGTTAATTACTCGC-GAGTGATTGAATGTGC
Gymnodinium_cf_pulchellum_Kawa    GAAAAC--TCCAGGGACATGGTAAGTCACTTCT-GGGTGACTGAAAGTGC
K_mikimotoi_Japan                 GAAAAC--TCTAAGGACATGGTAATTCGCTTCC-GAGTGACTGAATGTCC
K_mikimotoi_CCMP429               GAAAAC--TCTAAGGACATGGTAATTCGCTTCC-GAGTGACTGAATGTCC
K_brevis                          GAAAAC--TCTAGGGACATGGTAATTTGCTTCC-GGGCGACTGAATGTCC
KAPTB02                           GAAAAC--TCTAGGGACATGGTAATTTGCTTCC-GGGTGACTGAATGTGC
K_brevisulcata                    GAAAAC--TCTAGGGACTTGGTAATTTGCTTCC-GGGTGACTGAATGTCC
KPGB11                            GAAAAC--TCTAGGGACATGGTAATTTGCTTCC-GGGTGACTGAATGTGC
KUTN05                            GAAAAC--TCTGGGGACATGGTAATTTGCTTCC-GGGTGACTGAATGTGC
KULV01                            GAAAAC--TCTGGGGACATGGTAATTTGCTTCC-GGGTGACTGAATGTGC
Karenia_Parsons                   GAAAAC--TCTAGGGACATGGTAATTCGCTTCC-GGGTGGCTGAATGTGC
G._catenatum                      GACAAC--TCTTGGGACCTGGTAGCTCGCCCCT-GGGTGAGTGAATGTGC
G._nolleri                        GACAAC--TCTTGGGACCTGGTAGCTTGCCTCT-GGGTGAGTGAATGTGC
G._impudicum                      GACAAC--TCTTGGGACATGGTAGCTCGCCTCT-GGGTGAGCGAACGTGC
Gymnodinium trapeziforme          GATAAC--TCTTGGGACATGGTAGCTTCTCTGC-GGAGGGGTGAATGTGC
G._fuscum                         GATAAC--TCTTGGGACATGGTAGCTCGCCTCT-GGGCGAGTGAATGTGC
G.microreticulatumNC              GATAAC--TCTTGGGACATGGTAGCCTGCTTGC-GGGTGGGTGAATGTGC
G._palustre                       GATACC--TCTGGGGACATGTCAGCGAGCCTTC-GGGAGCGCGAATGTGC
G._cf._placidum                   GATACC--TCTGGGGACTTGNNNNNNNNNNNN-NNNNNNNNNNNNNNNN
Katodinium_cf_dorsalisulcum       GACAAC--TCTTGGGACATGGTAGCTCGCCTCT-GGGTGTGTGAACGTGC
Polyk_green                       GACAAT--TCTGGGGGCATGGTGGTCTGCCTCC-GGGTAGGTGAACGTGT
G._aureolum_S1                    GACAAC--TCTTGGGACATGGTAGCCTGCCCCT-GGGCGTGCGAATGTGC
G._aureolum_Adel                  GACAAC--TCTTGGGACATGGTAGCCTGCCCCT-GGGCGTGCGAATGTGC
G._chlorophorum                   GACAAC--CCTTGGGACATGGTAACTCGCCTCT-GGGTGTGTGAATGTGC
Lepid_cf_viride                   GACAAC--CCTTGGGACATGGTAACTCGCCTCT-GGGTGTGTGAAYGTGC
G._uncatenum                      GACAAC--CCCAAGGACATGGAAGGCCGCCCCC-GGGTGGCTGAAGGTGC
GUDE00                            GACAAC--CCCAAGGACATGGAAGGCCGCCCCC-GGGTGGCTGAAGGTGC
G._falcatum                       GAAAAC--TGTAGGGGCAAGGTAGCTCGTCTCC-GGGTGAGTGAATGCCC
GAPTB                             GAAGAC--TGCAGGGTCAAGGTATCTCGTCTCC-GGGTGAGTGAACGTCC
CPNU01                            GAAAAC--TGTAGGGTCAAGGTAGCTCGTCTCC-GGGTGAGTGAATGTCC
Akashiwo_sanguinea_NEP            GAAAAC--TCTGGGGACATGGATGCCAACCTCC-GGGGGGGTGAATGTGC
A._catenella_A3                   ATTACCTTTGCACATGAATGATAAGTCTCCTGTGGGGGGTG-GATTGCAT
CspTRA                            GAC-AACTCTTGGG-ACATGGTAACTTGCC-CACGGGTGAGCGAATGTGC
GY5HK                             GAT-ACCTCTTGGG-ACATGACAGCCTGCC-CCTGGGTGGGCGAATGTGC
GY5TRA                            GAT-ACCTCTTGGG-ACATGACAGCCTGCC-CCTGGGTGGGCGAATGTGC
WspKT01                           GATAGC--CTCAGGTTCATGATGTTCCACTGC--GGTGGAACTCCTGTGC
GICC53                            CGATGCTT-CTTGCCTTGTGCGTCATCGTCGGTTTGGAGGTGAGGACAAC
Contig[0004]                      ACGATGACGCACAAGGCAAGAAGCATCGCGCGCGGAAGGAT--CCAACCC
GISR01                            GACAAC--TCTTGGGACATGGTAGCTCGCCTCT-GGGTGAGCGAACGTGC
Gyro_spirale                      GAAA--ACTCAAGGGACATGGTAGTTTGCCCTC-GGGTGACCGAACGTGC
Gyro_rubrum                       GAAA--ACTCAAGGGACATGGTAGTTTGCCCAC-GGGTGACCGAACGTGC
Gyro_dominans                     GAAA--ACTCTGGGGTACTGGAAGCCACCTTTC-GGGGGGGTGAACGC-C
PSHK00                            GACGAC-CTCTCGGGACATGGAAGCTCGCCCCA-GGGTGTGCGAACGTGC
PKHK00                            GACGAC-CTCTCGGGACATGGAAGCTCGCCCCT-GGGTGTGCGAACGTAC
PSSH00                            GACGAC-CTCTCGGGACATGGAAGCTCGCCCCA-GGGTGTGCGAACGTGC
T_compacta_AY568562.1|            CATG--ATCCC----------------------------------------
T_jolla_AY455680.1|               CATG--ACGCC----------------------------------------
T_britannica_AY455679.1|          ACTG--CCCCCAACGATGTGCAATTTCGGCACT-TGCCGAATGACTGTTG
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                                601                                                650
                                 ↑                                                  ↑
Woloszynskia_pseudopalustris    TTGCAGGGACTCAGGCGTGTA--------CTTTGC----------------
Wolo_sp._CC                     TTGTAGGGACTCAGGCGTGTA--------CTTTGT----------------
K_micrum_Pth                    CTGGTAGAACTCATGTCTAAA--------CTGATTTCCGCATGTCTGGTC
Karlod_micrum_DB                CTGGTAGAACTCATGTCTAAA--------CTGATTTCCGCATGTCTGGTC
Taka_tasmanica_DW               TTGGCAGAACTCATTTCTGAA--------CTGAATTCCGCATGTCTGGTC
TTTL02                          TTGGCAGAACTCATTTCTGAA--------CTGAATTCCGCATGTCTGGTC
THNWB01                         TTGGCAGAACTCATTTCTGAA--------CTGAACTCCGCATGTCTGGTC
THPA01                          TTGGCAGAACTCATTTCTGAA--------CTGAACTCCGCATGTCTGGTC
TKSB                            CTGGCAGAACTCATTTCTGAA--------CTGATTCCTGCATGTCTGGTC
KDspGT03                        TTGGCAGAACTCATTTCTTAA--------CTGATTTCTGCATGTCTGGTC
KDTL11                          TTGGCAGAACTCATTTCTTAA--------CTGATTTCTGCATGTCTGGTC
Karenia_sp_Perth                TTGGTAGAACTCATTTCTGTA--------CTGATTCCTGCGTGTCTGGTC
K_bidigitata                    CTAGTTGAACTCATTTTTGAA--------CTGCTCCCTCTATGTCTGGTA
K_papilionacea_NZ               CTAGTTGAACTCATTTTTGAA--------CTGCTTTCTGTGTGTCTGGTA
K_selliformis                   CTAGTGGAACTCATTTTTGAA--------CTGCTTTT-GTATGTCTTGTT
Karenia_sp._Chile               CTAGTGGAACTCATTTTTGAA--------CTGCTTTTTGTATGTCTTGTT
Kd_micrum_NZ_U92257             CTGGTAGAACTCATGTCTAAA--------CTGATTTCCGCATGTCTGGTC
Gymnodinium_cf_pulchellum_Kawa  TTGGCAGAACTCATTTCTGAA--------CTGAATTCCGCATGTCTGGTC
K_mikimotoi_Japan               TCAGTTGAACTCATTTTTGAA--------CTGCTCTCTGTGTGTCTGGTA
K_mikimotoi_CCMP429             TCAGTTGAACTCATTTTTGAA--------CTGCTCTCTGTGTGTCTGGTA
K_brevis                        CCAGTTGAACTCATTTTTGAA--------CTGCTCACTGTGTGTCTGGTC
KAPTB02                         CTAGTTGAACTCATTTTTGAA--------CTGCTCCCTGTGTGTCTGGTA
K_brevisulcata                  CTAGTTGAACTCATTTTTGAA--------TTGCTTTTTATATGTCTTGTA
KPGB11                          CTAGTTGAACTCATTTTTGAA--------CTGCTTTCTGTGTGTCTGGTA
KUTN05                          CTAGTCGAACTCATTTGTGAA--------CTGCTCCCTGTGTGTCTGGTA
KULV01                          CTAGTCGAACTCATTTGTGAA--------CTGCTCCCTGTGTGTCTGGTA
Karenia_Parsons                 CTAGTTGAACTCATTTGTGAA--------CTGCTCCCTGTGTGTCTGGTA
G._catenatum                    CTTGCGGGACCCACCAACAAA--------CAGT-TCAACCTTT----GTG
G._nolleri                      CTTGCAGGACCCACCAACAAA--------CTGT-TCATCATTT----GTG
G._impudicum                    CAAGCAGAACGTACCCCCAGA--------CCTC-TCGTCCTT--------
Gymnodinium trapeziforme        CCTGTAGGACCCACCAGCGTA--------CTGTATTTTTT-TGTTTGGTT
G._fuscum                       CTTGTGGAATTTGCCACCATG--------TTGCGTCGACGTTGCGAGGTC
G.microreticulatumNC            CTTGTAGGACTCGCTTGTGTA--------CTGCAT-TCTTTTGTTTGGCT
G._palustre                     CCAGCAGTACTCGCTTGCGCA--------CAGTGCCCTCGCCT-----CA
G._cf._placidum                 NNNNNNNNNNNNNNNNNNNN--------NNNNNNNNNNNNNN-----NN
Katodinium_cf_dorsalisulcum     CTTGTAGAACTCATTCCCCTAGA------CTGTCAATTGAACGTGTGGTC
Polyk_green                     CTTGTAATACTCATCGCCATA--------CTTTTCAT-AAGTGTGAGGTC
G._aureolum_S1                  CTTGCAGAACTCACTGTCATAT-------TGCTCCTCCACATGTGAGGTC
G._aureolum_Adel                CTTGCAGAACTCACTGTCATAT-------TGCTCCTCCACATGTGAGGTC
G._chlorophorum                 CTTGTGGAACTCACCTTCGAA--------CTGCAAACCGAATGTGCGGTT
Lepid_cf_viride                 CTTGTGGAACTCACCTTCGAA--------CTGCAAATCGAATGTGCGGTT
G._uncatenum                    TTGGCGGAACTCAAGCGTGGA--------CT------------------
GUDE00                          TTGGCGGAACTCAAGCGTGGA--------CT------------------
G._falcatum                     TTGCAAGAAATCACGTGTGCG--------CTTGACATGGCGTGC------
GAPTB                           TTGTTGGAAGTCACGTGTATG--------CTGGATATGGCGTGC------
CPNU01                          CTGCAAGAAATCACGTGTGCG--------CTTGACATGGCGTGC------
Akashiwo_sanguinea_NEP          CTGGCAAGACCCGTCCTTGGA--------CTGTGA----------------
A._catenella_A3                 GTGCATGTAATGATTTGTGTT---------TTGATAAATG-TGTCTGGTG
CspTRA                          CTTGCAGAACTCACCTCCAAACTGATG-TCTCTTG---TTAGGT------
GY5HK                           CTTGTAGAACTCACTTGGAATCCGCGC-CCGTTGC---TCAGGT------
GY5TRA                          CTTGTAGAACTCACTTGGAATCCGCGC-CCGTTGC---TCAGGT------
WspKT01                         CTGCAGGGACTCAGGCTTGTA--------------------------------
GICC53                          --TCTTGGGACATGGTAGCTTGCCCCT--GGGCGAGCGAACGTGCCTTGT
Contig[0004]                    ACAGCACCGCTTGCGCTGACGCAGCGA-GAATCACCTTAGCACGCGACAA
GISR01                          CAAGCAGAACGTACCCCCAGAC-------CTCTCGTCCTTGTG-------
Gyro_spirale                    CTTGCAGAACTCATCTTTGAA--------TTGAAGCTCGTGTGTCTGGTT
Gyro_rubrum                     CTTGCAGAACTCACCTCTGGA--------TTGAAGACCTCATGTCCGGTT
Gyro_dominans                   CTTGTAGAACTCATCTCTAGA--------TTGAAGTGCTTGCGTTTGGTT
PSHK00                          CTTGCAGAACTTAGTCGTAGACTGGCGCTTTGCCCAAGCTGCGGGCCGTA
PKHK00                          CGTGCTTTTCCTTCTTTTAGACCGAC-CGCTGGCCCAGCCGTGGGCGGAA
PSSH00                          CTTGCAGAACTTAGTCGTAGACTGGCGCTCTGCCCAAGCTGCGGGCCGTA
T_compacta_AY568562.1|          --------ATTCACATGAAAAT-------TGCTTATAAGTGATTGAAAGT
T_jolla_AY455680.1|             --------ATTCACATGAAAGT-------CGCTTATAAGTGATTGAAAGT
T_britannica_AY455679.1|        CTGGGTGAGCTCATCTGTGATC-------TGAATCACTGTGAGTCTGGAT
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                                  651                                                                  700
                                   ▲                                                                    ▲
Woloszynskia_pseudopalustris      I----------------------------------------------------------------------I
Wolo_sp._CC                       ------------------------------------------------------------------------
K_micrum_Pth                      G-CAGTGTTC-TCATTACCTGCGTCTGG-GTTCGTGGCT--TGTAGCTTC
Karlod_micrum_DB                  G-CAGTGTTC-TCATNACCTGCGTCTGG-GTTCGTGGCT--TGTAGCTTC
Taka_tasmanica_DW                 G-CAGTGCTT-TCACCTCCTGGGCTTTG---------TT--CACTGCTCT
TTTL02                            G-CAGTGCTT-TCACCTCCTGGGCTTTG---------TT--CACTGCTCT
THNWB01                           G-CAGTGCTC-TCATTTCCCATGTCTCG---------TT--CGGAGCTTT
THPA01                            G-CAGTGCTC-TCATTTCCCATGTCTCG---------TT--CGGAGCTTT
TKSB                              G-CAGTGCTTTTCACTGCCTGTGTCTCG---------TT--CGGGGTGCC
KDspGT03                          G-CAGTGC-C-TCGTTGCCTGCGCTCGCTGCCTCGGGTC--TGGAGCTTC
KDTL11                            G-CAGTGC-C-TCGTTGCCTGCGCTCGCTGCCTCGGGTC--TGGAGCTTC
Karenia_sp_Perth                  G-CAGTGTCG-TCGCTCTCTCTCGGCTG---------TC--GGGTCCTT-
K_bidigitata                      G-CAGTGCTC-T-GCGTGTTCATCAGCACCATCATG-CT--GGCTCTGCC
K_papilionacea_NZ                 G-CAGTGCTC-T-GTGTGCTTGTCAGCAATCTCTTG-TT--AGT--TACC
K_selliformis                     G-CAGTGCTC-T-GCGTGCTTGCCTGTGATCCGGTG-CT--TGCTCTGCT
Karenia_sp._Chile                 G-CAGTGCTC-T-GTGTGCTTGCCTGTGATCTGGTG-CC--TGCTCTGCT
Kd_micrum_NZ_U92257               G-CAGTGTTC-T-CATTACCTGCGTCTGGGTTCGTGGCT--TGTAGCTTC
Gymnodinium_cf_pulchellum_Kawa    G-CAGTGCTT-T-CACTTCCCGTGCTTCGTTCGGAACTC--TCTGGGCTC
K_mikimotoi_Japan                 G-CACTGCTT-C-ATGTGCTTGCCTGCGATCT--T----------CTGCT
K_mikimotoi_CCMP429               G-CACTGCTT-C-ATGTGCTTGCCTGCGATCT--T----------CTGCT
K_brevis                          G-CACTGCTC-CCGTGTGCTTGCTTGCAATCT--T----------CTGCT
KAPTB02                           G-CAGTGCTC-T-GTGTGCTAGCCTGCAACCT--TGCA---AGCTCTGCC
K_brevisulcata                    G-CAGTGCTC-T-GTGAGCTTGCCTTCGATCTGATGCT---CGCTCTGCT
KPGB11                            G-CAGTGCTC-T-GTGTGCTTGTCAGCAATCTCTTGTT---AG--TTACC
KUTN05                            G-CAGTGCTC-TGCTGTGCATCTTCAAAAGCTTAAGCCTCGTCTCGTGCC
KULV01                            G-CAGTGCTC-TGCTGTGCATCTTCAAAAGCTTAAGCCTCGTCTCGTGCC
Karenia_Parsons                   G-CAGTGCTC-TGCTGGTTGCCTGTGG--GCTTTTGCT--GGCTC-TGTT
G._catenatum                      G-GGCTGCGT-TGCATCGTGGTGTGTGC-------------TCTTGCGA-
G._nolleri                        G-GGCTGCGT-TGCTTCGTGTTGCGTGC-------------TCTGGCGA-
G._impudicum                      ------------------------GTGC-----------------GGGA-
Gymnodinium trapeziforme          G-CGTTGCTGTGCTCTCCTCCGCTTACG-------------GTGGTGTG
G._fuscum                         G-AGTTGCCA-ATCGACAGCGCCACTTC-------------CATTGCGA-
G.microreticulatumNC              GCGC---------TGCTGTGCT----------------------TTGC---
G._palustre                       G-ATCGCGTT-GCTCTCTGCGAGCGTGC-------------GCGCATGT-
G._cf._placidum                   N-NNNNNNNN-NNNNNNNNNNNNNNNNN------------NNNNNNNN-
Katodinium_cf_dorsalisulcum       G-CGCTGTCG-TTCGCTGGCTTGCGCCC-------------GACGATCG-
Polyk_green                       G-TGCTGTC---TCGCTGCA----GACC-------------CACGGTC--
G._aureolum_S1                    G-TGCTGCCT-TTCTTTGGCACCTGTGT-------------TGGCGTTT-
G._aureolum_Adel                  G-TGCTGCCT-TTCTTTGGCACCTGTGT-------------TGGCGTTT-
G._chlorophorum                   G-CGCTGCCT-TTGCCGTGTGTCGCTGC-------------C---TCTC-
Lepid_cf_viride                   G-CGCTGCCC-TTGCTGTGCGTCGCTGC-------------CCCATCTC-
G._uncatenum                      ------------------------------------------------------------------------
GUDE00                            ------------------------------------------------------------------------
G._falcatum                       ------------------------------------------------------------------------
GAPTB                             ------------------------------------------------------------------------
CPNU01                            ------------------------------------------------------------------------
Akashiwo_sanguinea_NEP            G-CAGTGCTT-C-----------------------------------------
A._catenella_A3                   T-ATGTGTGT-GTGTTCCTGTGCTTGGG-------------GATGCTTCC
CspTRA                            --------T-------CTGTTGCCGTATACGG-CA---------------
GY5HK                             --------T-------CGCGTATCACGGATCT-CATTCGG----------
GY5TRA                            --------T-------CGCGTATCACGGATCT-CATTCGG----------
WspKT01                           ------------------------------------------------------------------------
GICC53                            G-GAACTCACCGCCAGA-------CTG-TGAATAGAATGTGTGGGAGCGT
Contig[0004]                      T-CTTACCAAGCAACACTGGTTCCATCCGCTTTCCTTTCAGCAAGTTCAG
GISR01                            ------------------------------------------------------------------------
Gyro_spirale                      G-CAATGCCT---------CCCTCTCTGGCTTGTCGCCC--TGGGAAATT
Gyro_rubrum                       G-CATTGCC----------CCCCATCAAGCTCGCTGCCC--TGGGAAATT
Gyro_dominans                     G-CAATGCCT---------CACTCTGT-GCCAGCTGCCC--TGGTAAATT
PSHK00                            GCCAGCGCAGGTGGTGTGAAAACAGCTTGCGTTGC---------CCTTT-
PKHK00                            GTCAGCGCACGCTGTGCTCAAATGGCGTGCGCGCG---------TGTTCT
PSSH00                            GCCAGCGCAGGTGGTGTGAAAACAGCTTGCGTTGC---------CCTTT-
T_compacta_AY568562.1|            GGTGGTAG-----------------------------------GAGCTCGC
T_jolla_AY455680.1|               GGTGGTAG-----------------------------------GAGCTTGT
T_britannica_AY455679.1|          GAAATCGTCTTGTGTCTCTCATCTGGTAGTACCATG-----TAACTTGGG
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                                  701                                                750
                                   ▲                                                  ▲
Woloszynskia_pseudopalustris      ----------------------------------------------------
Wolo_sp._CC                       ----------------------------------------------------
K_micrum_Pth                      TGTTACTCGTCGCGGGCTTCTGGGCCTGGTCG-TGGAGCATCTCCCTGAC
Karlod_micrum_DB                  TGTTACTCGTCGCGGGCTTCTGGGCCTGGTCG-TGGAGCATCTCCCTGAC
Taka_tasmanica_DW                 TAAGTGGGCCTCTGT--GCATGGGACAGGACG-TGGGGCATCTCCCTGAC
TTTL02                            TAAGTGGGCCTCTGT--GCATGGGACAGGACG-TGGGGCATCTCCCTGAC
THNWB01                           ------GCGCTCTG---ACCTGGGCTGGGGCG-TGGGGCATCTCCCTGAC
THPA01                            ------GCGCTCTG---ACCTGGGCTGGGGCG-TGGGGCATCTCCCTGAC
TKSB                              -TTGTGTGCTCTGTG----CTGGGCCGGGACG-TGGAGCATCTCCCTGAC
KDspGT03                          AGCTCTTCCTCGTTG--CTTTGAGCCTGGTCG-TGTGGCATCTCCCTGAC
KDTL11                            AGCTCTTCCTCGTTG--CTTTGAGCCTGGTCG-TGTGGCATCTCCCTGAC
Karenia_sp_Perth                  ------GCGACCTGC--GCA-GCTCTGAGGCGCTTTGGCACTTCCCTGAC
K_bidigitata                      TGGCAT-----GAGGGTT-----GCTGGTGCC-CAGAGCATGTCCTTGAC
K_papilionacea_NZ                 TGGCAT-----GAGACTT-----GCTGGCGCC-TGGAACACATCCTTGAC
K_selliformis                     TGGCAT-----G-GGGTT-----GCTGGTGCC-TGGAGCATGTCCTTGAC
Karenia_sp._Chile                 TGGCAT-----G-GGGTT-----GCTGGTGCC-TGGAGCATGTCCTTGAC
Kd_micrum_NZ_U92257               TGTTACTCGTCGCGGGCTTCTGGGCCTGGTCG-TGGAGCATCTCCCTGAC
Gymnodinium_cf_pulchellum_Kawa    TGAG---------------CTGGGCTGGGACG-TGGGGCATCTCCCTGAC
K_mikimotoi_Japan                 CTGCAT-----GAAGG-----TTGTTGGTGCC-AGGAGCATGTCCTTGAC
K_mikimotoi_CCMP429               CTGCAT-----GAAGG-----TTGTTGGTGCC-AGGAGCATGTCCTTGAC
K_brevis                          TGGCAT-----GAAGG-----T-GNTAATGCC-AGGAGCATGTCCTTGAC
KAPTB02                           TTGCAT-----TAGGA-----TTGCTGGTGCC-TGGAGCATGTCCTTGTC
K_brevisulcata                    TTGCAT-----G-GGA-----TTGTTGGTGCC-TGGAGCATGTCTTTGAC
KPGB11                            TGGCAT-----GAGAC-----TTGCTGGCGCC-TGGAACACATCCTTGAC
KUTN05                            TGGCTT-----TCGCTGT---CTGTCGGTGCC-TGGAGCACGTCCTTGAC
KULV01                            TGGCTT-----TCGCTGT---CTGTCGGTGCC-TGGAGCACGTCCTTGAC
Karenia_Parsons                   TGGCAT-----GAGTC-----TTGCTGGTGCC-CGGAGCACGTCCTTGAC
G._catenatum                      --GC--------TTCAC------------ACG-CGTGGCACCTTCCTTAC
G._nolleri                        --GC--------TTCAC------------ACG-CGTGGCACCTTCCTTAC
G._impudicum                      --GT--------TCTGC------------GCG-C--GGCA----------
Gymnodinium trapeziforme          GAGCGC----------------------------TTGCACCTTCCTTAC
G._fuscum                         --GCGGCATGTGTTGGT------------TCA-TAGGGCACCTTCCTGAC
G.microreticulatumNC              --TGTCCTCGTGGCTCTCAGCACTGGCACCTC-C-CTTACAATCCATG--
G._palustre                       --GC-------GCGCTT------------GCC-AGTGGCACCCCTCTGAT
G._cf._placidum                   --NN-------NNNNNN------------NNN-NNNNNNNNNNNNNNNNN
Katodinium_cf_dorsalisulcum       --CAAGGTCGTCGCGCCAAGC-------TAGC-CTCGGCACCTTCCGCAC
Polyk_green                       ----TGACTCACTCCTTGGGT--------TGCA-GTCGGCATCTCCCTGAC
G._aureolum_S1                    --GTGCCTGGCCTAGGTGTCG-------TTTG-CTGAGCACCTTCCTGAC
G._aureolum_Adel                  --GTGCCTGGCCTAGGTGTCG-------TTTG-CTGAGCACCTTCCTGAC
G._chlorophorum                   --GGCGGCTGGCGTGC-------------GCG-CGAGGCAC-TTCTGCAC
Lepid_cf_viride                   --GCCACTGTGCGTGCTGGGCGGTGCCGCGTG-CGGGGCACCTTCTGCAC
G._uncatenum                      ----------------------------------------GCTTGGCGCAA
GUDE00                            ----------------------------------------GCTTGGCGCAA
G._falcatum                       ------------------------------------CTCATTTGGTGAGC
GAPTB                             ------------------------------------CTCTTTTGGCGAGC
CPNU01                            ----------------------------------CTCATTTGGTAAGC
Akashiwo_sanguinea_NEP            ------------------------------------GGCATGTTTGCG--
A._catenella_A3                   T---------------------------TCCTTGGACTTACAAGCCCTGA
CspTRA                            ---------------C----------------------GT--TCTTGAC
GY5HK                             --GT---------ACC----------------------AC--CCTGAAC
GY5TRA                            --GT---------ACC----------------------AC--CCTGAAC
WspKT01                           ----------------------------------------------------
GICC53                            TGCTCGT--CGCGCTGTC-----------------------------G
Contig[0004]                      GCACTTTTAACTCTCTTTTCAAAG---TCCTTTTCATCTTTCCCTCATGG
GISR01                            ----------------------------------CGGGAGTTCTGC
Gyro_spirale                      CAGCAGGAGCGCAGCCTTTTATGGCGA--------GAGCAT-TCCTTGAC
Gyro_rubrum                       CAGCAGGTGCGCGGC-TTTGCTGGCGG--------GAGCAC-TCCTTGAC
Gyro_dominans                     CAGCAGGTGCGCGGCGCTCATCAGTGG--------GTGCGT-TCCTTGAC
PSHK00                            -GTTACGAGGGGC------GCTGGTTTACGC---CCGACTATTGGTGCAC
PKHK00                            CGTCACGAGGACT------GCTAGCTTCCGCT--CCGACCCTCGGTGCTT
PSSH00                            -GTTACAAGGGGC------GCTGGTTTACGC---CCGACTATTGGTGCAC
T_compacta_AY568562.1|            TGCCGTTCTGTTGATTGG----------------TGTGCCCCGCGGGCAC
T_jolla_AY455680.1|               TGCCGTTCTGTTGATTGG----------------TGTGCCCTTCGGGCAC
T_britannica_AY455679.1|          CTGCATGATCAACTGCCT----------------GTGGCACATCCCAGAC
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                                                751                                                800
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Woloszynskia_pseudopalustris                    ----------GTGACAAAATGGTTCTCTTCGACCCGTCTTGAAACACGGA
Wolo_sp._CC                                     ----------GTGACAAAATGGTTCTCTTCGACCCGTCTTGAAACACGGA
K_micrum_Pth                                    ATTAGT-ACGATGACAAAATGGTTTCATTCGACCCGTCTTGAAACACGGA
Karlod_micrum_DB                                ATTAGT-ACGATGACAAAATGGTTTCATTCGACCCGTCTTGAAACACGGA
Taka_tasmanica_DW                               ATCAGT-ACGATGACAAAATGGTTTCATTCGACCCGTCTTGAAACACGGA
TTTL02                                          ATCAGT-ACGATGACAAAATGGTTTCATTCGACCCGTCTTGAAACACGGA
THNWB01                                         ATCAGT-ACGATGACAAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
THPA01                                          ATCAGT-ACGATGACAAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
TKSB                                            ATAAGT-ACGATGACAAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
KDspGT03                                        ATAAGT-ACGATGACAAAATGGTTTCATTCGACCCGTCTTGAAACACGGA
KDTL11                                          ATAAGT-ACGATGACAAAATGGTTTCATTCGACCCGTCTTGAAACACGGA
Karenia_sp_Perth                                ACAAGT-ACGATGACAAAATGGTTTCATTCGACCCGTCTTGAAACACGGA
K_bidigitata                                    ATAAGT-ACGATGACGAAATGGTTTTATTCGACCCG--------------
K_papilionacea_NZ                               ATAAGTTACGATGACGAAATGGTTTTATTCGACCCG--------------
K_selliformis                                   ATAAGT-ACGATGACGAAATGGTTTTATTCGACCCG--------------
Karenia_sp._Chile                               ATAAGT-ACGATGACAAAANNGNTTT-----------------------
Kd_micrum_NZ_U92257                             ATTAGT-ACGATGACAAAATGGTTTCATTCGACCCG--------------
Gymnodinium_cf_pulchellum_Kawa                  ATCAGT-ACGATGACAAAATGGTTTTATTCGACCCG--------------
K_mikimotoi_Japan                               ATTAGA-ACGATGACGAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
K_mikimotoi_CCMP429                             ATTAGA-ACGATGACGAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
K_brevis                                        ATAAGA-ACGATGACGAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
KAPTB02                                         ATAAGT-ACGATGACGAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
K_brevisulcata                                  ATAAGT-ACGATGACGAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
KPGB11                                          ATAAGT-ACGATGACGAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
KUTN05                                          ATAAGT-ACGATGACGAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
KULV01                                          ATAAGT-ACGATGACGAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
Karenia_Parsons                                 ATAAGT-ACGATGACAAAATGGTTTTATTCGACCCGTCTTGAAACACGGA
G._catenatum                                    AAGCTA-ATGGTGACGAAATGGTTCTATTCGACCCGTCTTGAAACACGGA
G._nolleri                                      AAGCAA-ATGGTGACGAAATGGTTCTATTCGACCCGTCTTGAAACACGGA
G._impudicum                                    --------TGGTGACTGAATCCTTCTATTCGACCCGTCTTGAAACACGGA
Gymnodinium trapeziforme                        AATGTT--TGGTGACGAAATGGTTCTATTCGACCCGTCTTGAAACACGGA
G._fuscum                                       AA--CA-ACGGTGACCAAATGGTTCTTTTCGACCCGTCTTGAAACACGGA
G.microreticulatumNC                            ----------GTGACGAAATGGTTCTATTCGACCCGTCTTGAAACACGG-
G._palustre                                     GCATCT-CTAGTGGCGAAATGGTTCCATTCGACCCGTCTTGAAACACGGA
G._cf._placidum                                 NNNNNN-NNNNNNNNNNNNNNNNNNNNNNNACCCGTCTTGAAACACGGA
Katodinium_cf_dorsalisulcum                     ATCGCC-ATGGTGACTAAATGGTTCTATTCGACCCGTCTTGAAACACGGA
Polyk_green                                     ATATCG-ATGGTGGC-----------------------------------
G._aureolum_S1                                  ATAGCC-ATGGTGACTAAATGGTTCTATTCGACCCGTCTTGAAACACGGA
G._aureolum_Adel                                ATAGCC-ATGGTGACTAAATGGTTCTATTCGACCCGTCTTGAAACACGGA
G._chlorophorum                                 ACAGCT-ATGGTGACTAAATGGTTCTATTCGACCCGTCTTGAAACACGGA
Lepid_cf_viride                                 ACAGCT-ATGGTGACTAAATGGTTCTATTCGACCCGTCTTGAAACACGGA
G._uncatenum                                    CGCGCCTGCGCTGACGACATGGATTCGTTCGGCCCGTCTTGAAACACGGA
GUDE00                                          CGCGCCTGCGCTGACGACATGGATTCGTTCGGCCCGTCTTGAAACACGG-
G._falcatum                                     CACAAGATTGATGACAAAATGGTTCCATACGACCCGTCTTGAAACACGGA
GAPTB                                           CACAAAATCGATGACTAAATGGTTCTATCCGACCCGTCTTGAAACACGGA
CPNU01                                          CACAAGATTGATGACAAAATGGTTCCACACGACCCGTCTTGAAACACGGA
Akashiwo_sanguinea_NEP                          -------GTGCTGACGAAATGCTTCTATTCGACCCGTCTTGAAACACGGA
A._catenella_A3                                 CACACACATGCTGGCAAAATGCTTCTGCTTGACCCGTCTTGAAACACGGA
CspTRA                                          AAA-GC-GTGGTGACAGAATGGTTCTATTCGACCCGTCTTGAAACACGGA
GY5HK                                           ACATGC-ATGGTGACTGAATGGTTCTATTCGACCCGTCTTGAAACACGG-
GY5TRA                                          ACATGC-ATGGTGACTGAATGGTTCTATTCGACCCGTCTTGAAACACGG-
WspKT01                                         --CAGT--TGGTGACGAAATGGTTCTCTTCGACCCGTCTTGAAACACGGA
GICC53                                          CCCGT--GGGATATA---AGGCTGCGGCAGTGCGCGCG-C---GCACCTT
Contig[0004]                                    TACTTGTTTGCTATCGGTTTCGAACCCATATTTAGCTTGGGATGAAATTT
GISR01                                          GCGCGGCATGGTGACTGAATGGTTCTATTCGACCCGTCTTGAAACACGGA
Gyro_spirale                                    GCGCAG-ATGGTGACAAAATGGTTTCACTCGACCCGTCTTGAAACACGGA
Gyro_rubrum                                     GCGAAG-ATGGTGACAAAATGGTTTCACTCGACCCGTCTTGAAACACGGA
Gyro_dominans                                   GCTACG-ATGCTGACAAAATGGTTTCACTCGACCCGTCTTGAAACACGGA
PSHK00                                          -TTGCT-TTTGTGACTGAATGGTTCTATTCGACCCGTCTTGAAACACGG-
PKHK00                                          -TAGCT-TTTGTGACT-AATGGTTCTCTTCGACCCGTCTTGAAACACGG-
PSSH00                                          -TTGCT-TTTGTGACTGAATGGTTCTATTCGACCCGTCTTGAAACACGG-
T_compacta_AY568562.1|                          GCTTGG---GATGACAAAATGGTTCTATTTGACCCGTCTTGAAACACGGA
T_jolla_AY455680.1|                             GCTGGG---GATGACCAAAATGGTTCTATTTGACCCGTCTTGAAACACGGA
T_britannica_AY455679.1|                        GCAATT---CATGTCGTAACGGCTTCTTTCGACCCGTCTTGAAACACGGA
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                                  801                                                 850
                                   ↑                                                   ↑
Woloszynskia_pseudopalustris      CCAAGGAGTCTAGCACATGTGCGAGTTCACAGGTGGCAAG--CCTGCTTG
Wolo_sp._CC                       CCAAGGAGTCTAGCACATGTGCGAGTTCACAGGTGGCAAG--CCTGCTTG
K_micrum_Pth                      CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGATTG
Karlod_micrum_DB                  CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGATTG
Taka_tasmanica_DW                 CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGCTTG
TTTL02                            CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGCTTG
THNWB01                           CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGCTTG
THPA01                            CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGCTTG
TKSB                              CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGCTTG
KDspGT03                          CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGATTG
KDTL11                            CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTTAAA--CCTGATTG
Karenia_sp_Perth                  CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGTCAAA--CCTGCTTG
K_bidigitata                      --------------------------------------------------
K_papilionacea_NZ                 --------------------------------------------------
K_selliformis                     --------------------------------------------------
Karenia_sp._Chile                 --------------------------------------------------
Kd_micrum_NZ_U92257               --------------------------------------------------
Gymnodinium_cf_pulchellum_Kawa    --------------------------------------------------
K_mikimotoi_Japan                 CCAAGGAGTCTAACATATGTGCAAGTTCACGGGCGGGAAAA-CCTGCTTG
K_mikimotoi_CCMP429               CCAAGGAGTCTAACATATGTGCAAGTTCACGGGCGGGAAAA-CCTGCTTG
K_brevis                          CCAAGGAGTCTAACATATGTGCAAGTTCACGGGCGGGAAAA-CCTGCTTG
KAPTB02                           CCAAGGAGTCTAACATATGTGCAAGTTCACGGGCGGGAAAA-CCTGCTTG
K_brevisulcata                    CCAAGGAGTCTAACATATGTGCAAGTTCACGGGCGGGAAAA-CCTGCTTG
KPGB11                            CCAAGGAGTCTAACATATGTGCAAGTTCACGGGCGGGAAAA-CCTGCTTG
KUTN05                            CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGGTAAA--CCTTCTTG
KULV01                            CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGGTAAA--CCTTCTTG
Karenia_Parsons                   CCAAGGAGTCTAACATATGTGCAAGTTCACGGGTGGGAAAA-CCTTCTTG
G._catenatum                      CCAAGGAGTCTAACATGTGTGCGAGTTCATGGGTGGTAAAA-CTTGCGTG
G._nolleri                        CCAAGGAGTCTAACATATGTGCGAGTTCATGGGTGGTAAAA-CTTGCGTG
G._impudicum                      CCCCGGAGTCTAACATATGTGCAAGTTCTTGGGTGGTGAAA-CCTACGTG
Gymnodinium trapeziforme          CCAAGGAGTCTAACATATGTGCGAGTTCATGGGCGTTAAA--CCTATGTG
G._fuscum                         CCAAGGAGTCTAACATACGTGCGAGTTCCTGGGTGGAAAAAACCTACCCG
G.microreticulatumNC              --------------------------------------------------
G._palustre                       CCAAGGAGTCTAACATGTGTGCGAGTTCTTGGGTGTCAAG--CCTACGTG
G._cf._placidum                   CCAAGGAGTCTAACATGTGTGCGAGTTCTTGGGTGTCAAG--CCTACGTG
Katodinium_cf_dorsalisulcum       CCAAGGAGTCTAACATATGTGCGAGTTCTTGGGTT-TGAAA-CCTACGTG
Polyk_green                       --------------------------------------------------
G._aureolum_S1                    CCAAGGAGTCTAACATGTGTGCGAGTTCTTGGGCGGCGAAA-CCTACGTG
G._aureolum_Adel                  CCAAGGAGTCTAACATGTGTGCGAGTTCTTGGGCGGCGAAA-CCTACGTG
G._chlorophorum                   CCAAGGAGTCTAACATATGTGCGAGTTCTTGGGTGGCGAAA-CCTACGTG
Lepid_cf_viride                   CCAAGGAGTCTAACATATGTGCGAGTTCTTGGGTGGCGAAA-CCTACGTG
G._uncatenum                      CCAAGGAGTCTAGCATATGTGCAAGCACATGGGTGTT-AAA-CCTAAGTG
GUDE00                            --------------------------------------------------
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GAPTB                             CCAAGGAGTCTAGCATATGTGCAAGTTCACGGGTGTT-AAA-CCTGTTTG
CPNU01                            CCAAGGAGTCTAGCGTATGTGCAAGTTCACGGGTGTC-AAA-CCTGTTTG
Akashiwo_sanguinea_NEP            CCGAGGAGTCTAACATGTGTGCGAGCTCACGGGTGTCAAA--CCTGCTTG
A._catenella_A3                   CCAAGGAGTCTAACAGATGTGCAAAGTTTGAAGGTGGTATA-CCTTTGTG
CspTRA                            CCAAGGAGTCTAACATATGTGCAAGTACTTGGGTGGCGAA-ACCTATGTG
GY5HK                             --------------------------------------------------
GY5TRA                            --------------------------------------------------
WspKT01                           CCAAGGAGTCTAGCACATGTGCGAGTTCACAGGTGGCAAG--CCTGCTTG
GICC53                            TCACA-CACAACCATGGTGACTAAATGGTTCCATTCGACCCGTCTTGAAA
Contig[0004]                      ACCACCCAATTTGCGCTCCAATC-CCGAGGAACGTGACTCTAAGAACGTA
GISR01                            CCAAGGAGTCTAACATATGTGCAAGTTCTTGGGTGGTGAAA-CCTACGTG
Gyro_spirale                      CCAAGGAGTCTAACATATGTGCAAGTTCCCGGGTGG-CCAAACCTGCTGG
Gyro_rubrum                       CCAAGGAGTCTAACATATGTGCAAGTTCCCGGGTGG-CCAAACCTGCTGG
Gyro_dominans                     CCAAGGAGTCTAACATATGTGCAAGTTCCCGGGTGG-TTAAACCTGTTGG
PSHK00                            --------------------------------------------------
PKHK00                            --------------------------------------------------
PSSH00                            --------------------------------------------------
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T_britannica_AY455679.1|          CCAAGGAGTTTGACGCATCTGCAAGTTCATGACTGG-ACAAACTTATGTA
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                                  851                                                                  900
                                   ▲                                                                    ▲
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Wolo_sp._CC                       CGCAATGAAGGTGACAGCTGGGATCT---CTGCACCAGCAACCGACCAAT
K_micrum_Pth                      CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
Karlod_micrum_DB                  CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
Taka_tasmanica_DW                 CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
TTTL02                            CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
THNWB01                           CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
THPA01                            CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
TKSB                              CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
KDspGT03                          CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
KDTL11                            CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCGACCAAT
Karenia_sp_Perth                  CGCAATGAAAGTGAY-GCTGGGATTT---CTGCACCAGCAACCGACCAAT
K_bidigitata                      --------------------------------------------------
K_papilionacea_NZ                 --------------------------------------------------
K_selliformis                     --------------------------------------------------
Karenia_sp._Chile                 --------------------------------------------------
Kd_micrum_NZ_U92257               --------------------------------------------------
Gymnodinium_cf_pulchellum_Kawa    --------------------------------------------------
K_mikimotoi_Japan                 CGCAATGAAAGTGACTGCTGGGATTT---TTGCACCAGCAACCGACCAAT
K_mikimotoi_CCMP429               CGCAATGAAAGTGACTGCTGGGATTT---TTGCACCAGCAACCGACCAAT
K_brevis                          CGCAATGAAAGTGACTGCTGGGATAT---CTGCACCAGCAACCGACCAAT
KAPTB02                           CGCAATGAAAGTGACTGCTGGGATAT---CTGCACCAGCAACCGACCAAT
K_brevisulcata                    CGCAATGAAAGTGACTGCTGGGATAT---CTGCACCAGCAACCGACCAAT
KPGB11                            CGCAATGAAAGTGACTGCTGGGATCT---CTGCACCAGCAACCGACCAAT
KUTN05                            CGCAATGAAAGTGACTGCTGGGATAT---CTGCACCAGCAACCGACCAAT
KULV01                            CGCAATGAAAGTGACTGCTGGGATAT---CTGCACCAGCAACCGACCAAT
Karenia_Parsons                   CGCAATGAAAGTGACTGCTGGGATAT---TTGCACCAGCAACCAACCAAT
G._catenatum                      CGCAATGAAAGTGATTGCTGAGACCT---TAGCATCAGCGGCCGACCAAT
G._nolleri                        CGCAATGAAAGTGATTGCTGAGATAT---TAGCATCAGCGGCCGACCAAT
G._impudicum                      CGCAATGAAAGTGATTACTGAGATGC---TTGCATCAGCAGCCGACCGAT
Gymnodinium trapeziforme          CGTAATGAAAGTGAGTGCTGAGATGC---TTGCATCAGCAGCCGACCGAT
G._fuscum                         CGCAATGAAAGTGAGTGCTGAGATCC---TTGCATCAGCAGCCGACCGAT
G.microreticulatumNC              --------------------------------------------------
G._palustre                       CGCAATGAAAGTGAGTACTGAGATGT---TCGCATCAGCAGCCGACCGAT
G._cf._placidum                   CGCAATGAAAGTGAGTACTGAGATGT---TTGCATCAGCAGCCGACCGAT
Katodinium_cf_dorsalisulcum       CGCAATGAAAGTGATTACTGAGAATC---TAGCATCAGCAGCCGACCGAT
Polyk_green                       --------------------------------------------------
G._aureolum_S1                    CGCAATGAAAGTGATTACTGAGATGC---TAGCATCAGCAGCCGACCGAT
G._aureolum_Adel                  CGCAATGAAAGTGATTACTGAGATGC---TAGCATCAGCAGCCGACCGAT
G._chlorophorum                   CGCAATGAAAGTGAGTACTGAGATCT---TTGCATCAGCAGCCGACCGAT
Lepid_cf_viride                   CGCAATGAAAGTGAGTACTGAGATCT---TTGCATCAGCAGCCGACCGAT
G._uncatenum                      CGCAATGAAAGTGACTGCTGGGATCCC--TTGCACCAGCAACCGACTGAT
GUDE00                            --------------------------------------------------
G._falcatum                       CGTAATGAAAGTGACTGCTGGGATTT---CTGCACCAGCAACCGACCAAT
GAPTB                             CGTAATGAAAGTGACTGCTGGGATTC---CTGCACCAGCAACCGACCAAT
CPNU01                            CGTAATGAAAGTGACTGCTGGGATTT---CTGCACCAGCAACCGACCAAT
Akashiwo_sanguinea_NEP            CGCAATGAAAGTGACTGCTGGGATAC---TTGCACCAGCAACCGATCTGT
A._catenella_A3                   TGCAATGAAAGTGTCAGCTGGGATAT---GTGCACCAGCACCCAATTAAT
CspTRA                            CGCAATGAAAGTGAGTGCTGAGATGC---TTGCATCAGCAGCCGACCAAT
GY5HK                             --------------------------------------------------
GY5TRA                            --------------------------------------------------
WspKT01                           CGCAATGAAGGTGACAGCTGGGATTT---CTGCACCAGCAACCGACCAAT
GICC53                            CACGG-----------------------------------------------
Contig[0004]                      CGCCGTGCACGGAGGTCTGCGAGTGAC--AAACAGGATTCTCACCCTCAA
GISR01                            CGCAATGAAAGTGATTACTGAGATGC---TTGCATCAGCAGCCGACCGAT
Gyro_spirale                      CGTAATGAAAGTGACTGCTGGGATTT---TTGCACCAGCAACCGACTGAC
Gyro_rubrum                       CATAATGAAAGTGACTGCTGGGATCT---TTGCACCAGCAACCGACCAAT
Gyro_dominans                     CGTAATGAAAGTGACTGCTGGGATTT---TTGCACCAGCAACCGACTGAC
PSHK00                            --------------------------------------------------
PKHK00                            --------------------------------------------------
PSSH00                            --------------------------------------------------
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T_britannica_AY455679.1|          CGCAATGAAAATAACAGCTGGGAGGTACGTCGCGCCAGCAACTGATGTGC
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                                 901                                                 950
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Wolo_sp._CC                      CAATTGAGAGAAGTTTGAGTATGAGCATATGTGTTAGGACCCGAAAGATG
K_micrum_Pth                     CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
Karlod_micrum_DB                 CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
Taka_tasmanica_DW                CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
TTTL02                           CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
THNWB01                          CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
THPA01                           CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
TKSB                             CAATTGTGTGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
KDspGT03                         CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
KDTL11                           CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
Karenia_sp_Perth                 CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
K_bidigitata                     ----------------------------------------------------
K_papilionacea_NZ                ----------------------------------------------------
K_selliformis                    ----------------------------------------------------
Karenia_sp._Chile                ----------------------------------------------------
Kd_micrum_NZ_U92257              ----------------------------------------------------
Gymnodinium_cf_pulchellum_Kawa   ----------------------------------------------------
K_mikimotoi_Japan                CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
K_mikimotoi_CCMP429              CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
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KAPTB02                          CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
K_brevisulcata                   CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
KPGB11                           CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
KUTN05                           CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
KULV01                           CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
Karenia_Parsons                  CAATTGTGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
G._catenatum                     CAATTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
G._nolleri                       CAATTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
G._impudicum                     CAATTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
Gymnodinium trapeziforme         CGATTGGGAGANGTTTGAGCATGAGCATATCTGTTANGACCCGAAAGATG
G._fuscum                        CAATTGGGAGAGGTTTGAGCAAGAGCACGCCTGTTAGGACCCGAAAGATG
G.microreticulatumNC             ----------------------------------------------------
G._palustre                      CGATTGGGAGAGGTTTGAGCAAGAGCATATCTGTTAGGACCCGAAAGATG
G._cf._placidum                  CGATCGGGAGAGGTTTGAGCAAGAGCATATCTGTTAGGACCCGAAAGATG
Katodinium_cf_dorsalisulcum      CAATTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
Polyk_green                      ----------------------------------------------------
G._aureolum_S1                   CAATTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
G._aureolum_Adel                 CAATTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
G._chlorophorum                  CGACTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
Lepid_cf_viride                  CAACTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
G._uncatenum                     CAATTGGGAGAGGTTTGAGTTTGAGCATATCTGTTAGGACCCGAAAGATG
GUDE00                           ----------------------------------------------------
G._falcatum                      CAATTGAGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
GAPTB                            CAATTGAGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
CPNU01                           CAATTGAGAGAGGTTTGAGTATGAGCATATCTGTTAGGACCCGAAAGATG
Akashiwo_sanguinea_NEP           CAACTGAGAGAGATTTGAGTTTGAGCATACCTGTTAGGACCCGAAAGATG
A._catenella_A3                  TAGCGGACAGAGGTTTGAGTTGGAGCATATCTATTAGGACCCGAAAGATG
CspTRA                           CAATTGGGAGAGGTTTGAGCATGAGCATATCTGTTAGGACCCGAAAGATG
GY5HK                            ----------------------------------------------------
GY5TRA                           ----------------------------------------------------
WspKT01                          CAATTGAGAGAAGTTTGAGTTTGAGCATATGTGTTAGGACCCGAAAGATG
GICC53                           ----------------------------------------------------
Contig[0004]                     TGATTCTCTTTTCCAAGAGGC-TTTCACCCGCGCCCCCGCTAGCAGTACA
GISR01                           CAATTGGGAGAGGTTTGAGCATGAGCATAT--------------------
Gyro_spirale                     CAATTGAGAGAGGTTTGAGTATGAGCACATCTGTTAGGACCCGAAAGATG
Gyro_rubrum                      CGACTTTGAGAGGTTTGAGTATGAGCACATCTGTTAGGACCCGAAAGATG
Gyro_dominans                    CAATTGAGAGAGGTTTGAGTATGAGCACATCTGTTAGGACCCGAAAGATG
PSHK00                           ----------------------------------------------------
PKHK00                           ----------------------------------------------------
PSSH00                           ----------------------------------------------------
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T_jolla_AY455680.1|              CAAGTGAGAGAGGTTTGAGTGTGAGCATGCGTGCCAGGACCCGAAAGATG
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Wolo_sp._CC                     GTGAACTATGCCTGCGAAGGGCAAAGT----------------------
K_micrum_Pth                    GTGAACTATGCGTGAGAAGGGTGAAGTCCAGGGG----------------
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Taka_tasmanica_DW               GTGAACTATGCCTGAGAAGGGTGAAGTCAGGGG----------------
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Karenia_sp_Perth                GTGAACTATGCCTGTG----------------------------------
K_bidigitata                    ------------------------------------------------
K_papilionacea_NZ               ------------------------------------------------
K_selliformis                   ------------------------------------------------
Karenia_sp._Chile               ------------------------------------------------
Kd_micrum_NZ_U92257             ------------------------------------------------
Gymnodinium_cf_pulchellum_Kawa  ------------------------------------------------
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K_mikimotoi_CCMP429             GTGAACTATGCTTGAGAAGGGTGAAGTCAGGGGAAACTCTGATGGAGGCT
K_brevis                        GTGAACTATGCTTGAGAAGGGTGAAGTCAGGGGAAACTCTGATGGAGGCT
KAPTB02                         GTGAACTATGCTTGAGAAGGGTGAAGTCAGGGGAAACTCTGATGG-----
K_brevisulcata                  GTGAACTATGCCTGAGAAGGGTGAAGTCC--------------------
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KUTN05                          GTGAACTATGCCTGAGAAGGGTGAAGTCAGGGG----------------
KULV01                          GTGAACTATGCCTGAGAAGGGTGAAGTCAGGGGAAACTCTGATGGAGGC-
Karenia_Parsons                 GTGAACTATGCCTGAGAAGGGTGAAGTCAGGGG----------------
G._catenatum                    GTGAACTATGCCTGAGAAGGGTGAAGTCTGGGGAAACTCAGATGGAGGCT
G._nolleri                      GTGAACTATGCCTGAGAAGGGTGAAGTCTGGGGAAACTCAGATGGAGGCT
G._impudicum                    GTGAACTATGCCTGTGAAGGGTGAAGTCTGGGGAAACTCAGATGGAGGCT
Gymnodinium trapeziforme        GTGAACTATGCCTGAGAAGGGTGAAGTCTGGGGAAACTCAGATGGAGGCT
G._fuscum                       GTGAACTATGCCTGAGAAGGGCGAAGTCTGGGGAAACTCAGGTGGAGGCT
G.microreticulatumNC            ------------------------------------------------
G._palustre                     GTGAACTATGCCTGAGAAGGGCAAAGTCTGGGGAAACTCAGATGGAGGCT
G._cf._placidum                 GTGAACTATGCCTGGGAAGGGCAAAGTCTGGGGAAACTCAGATGGAGGCT
Katodinium_cf_dorsalisulcum     GTGAACTATGCCTGTGAAGGGCGAAGTCTGGGGAAACTCAGATGG-----
Polyk_green                     ------------------------------------------------
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G._aureolum_Adel                GTGAACTATGCTTGTGAAGGGTGAAGTCTGGGGA----------------
G._chlorophorum                 GTGAACTATGCCTGTGAAGGGTGAAGCCTGGGGAAACTCAGGTGGAGGCT
Lepid_cf_viride                 GTGAACTATGCCTGTGAAGGGTGAAGCCTGGGG----------------
G._uncatenum                    GTGAACTATGCCTGAGAAGAACAAA------------------------
GUDE00                          ------------------------------------------------
G._falcatum                     GTGAACTATGCCTGAGAAGGGCAAACTCAGGGGA----------------
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CspTRA                          GTGAACYATGCCTGAGAAGGGTGAAGTCTGGGGAAACTCAGA--------
GY5HK                           ------------------------------------------------
GY5TRA                          ------------------------------------------------
WspKT01                         GTGAACTATGCCTGCGAAGGGCAAAGTCAGGGGAAACTCTGATGGA----
GICC53                          ------------------------------------------------
Contig[0004]                    TCTTGAGATTACAATTCACA-GCCTGAGGCCGCAATTTCAATGTTGAGCT
GISR01                          ------------------------------------------------
Gyro_spirale                    GTGAACTATGCCTGAGAAGGGTGAAGTCAGGGGAAACTCTGATGGAGGCT
Gyro_rubrum                     GTGAACTATGCCTGAGAAGGGTGAAGTCAGGGGAAACTCTGATGGAGGCT
Gyro_dominans                   GTGAACTATGCCTGAGAAGGGTGAAGTCAGGGGAAACCCTGATGGAGGCT
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PKHK00                          ------------------------------------------------
PSSH00                          ------------------------------------------------
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T_britannica_AY455679.1|        GTGAACTATGCTTGAGAAGGGCAAACTCAGGGGAAACTCTGATGGAGGCT
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K_micrum_Pth                    --------------------------------------------------
Karlod_micrum_DB                CGTAGCGATACTGACGTGCAAATCGTTCGTCATACTTGCGTATAGGGGCG
Taka_tasmanica_DW               --------------------------------------------------
TTTL02                          --------------------------------------------------
THNWB01                         --------------------------------------------------
THPA01                          --------------------------------------------------
TKSB                            --------------------------------------------------
KDspGT03                        --------------------------------------------------
KDTL11                          --------------------------------------------------
Karenia_sp_Perth                --------------------------------------------------
K_bidigitata                    --------------------------------------------------
K_papilionacea_NZ               --------------------------------------------------
K_selliformis                   --------------------------------------------------
Karenia_sp._Chile               --------------------------------------------------
Kd_micrum_NZ_U92257             --------------------------------------------------
Gymnodinium_cf_pulchellum_Kawa  --------------------------------------------------
K_mikimotoi_Japan               CGTAGCGATACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
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K_brevisulcata                  --------------------------------------------------
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KUTN05                          --------------------------------------------------
KULV01                          --------------------------------------------------
Karenia_Parsons                 --------------------------------------------------
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G._nolleri                      CGTAGCGATACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
G._impudicum                    CGTAGCGATACTGACGTGCAAATCGTTCGTCATACATGGGTATAGGGGCG
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G._fuscum                       CGTAGCGATACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
G.microreticulatumNC            --------------------------------------------------
G._palustre                     CGTAGCGATACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
G._cf._placidum                 CGTAGCGATACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
Katodinium_cf_dorsalisulcum     --------------------------------------------------
Polyk_green                     --------------------------------------------------
G._aureolum_S1                  CGTAGCGATACTGACGTGCAAATCGTTCGTCATACATGGGTATAGGGGCG
G._aureolum_Adel                --------------------------------------------------
G._chlorophorum                 CGTAGCGATACTGACGTGCAAATCGTTCGTCATACATGGGTATAGGGGCG
Lepid_cf_viride                 --------------------------------------------------
G._uncatenum                    --------------------------------------------------
GUDE00                          --------------------------------------------------
G._falcatum                     --------------------------------------------------
GAPTB                           --------------------------------------------------
CPNU01                          --------------------------------------------------
Akashiwo_sanguinea_NEP          CGTAGCGATACTGACGTGCAAATCGTTCGTCAAACATGAGTATAGGGGCG
A._catenella_A3                 CGTATCGATACTGACGTGCAAATCGTTCGTCATATTTGGGTATAGGGGCG
CspTRA                          --------------------------------------------------
GY5HK                           --------------------------------------------------
GY5TRA                          --------------------------------------------------
WspKT01                         --------------------------------------------------
GICC53                          --------------------------------------------------
Contig[0004]                    CATCCCTGTTCATTCGCCATTACTAAGGGAATCCTG----------
GISR01                          --------------------------------------------------
Gyro_spirale                    CATAGCGATACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
Gyro_rubrum                     CATAGCGATACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
Gyro_dominans                   CATAGCGATACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
PSHK00                          --------------------------------------------------
PKHK00                          --------------------------------------------------
PSSH00                          --------------------------------------------------
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T_jolla_AY455680.1|             TGCAGCGCTACTGACGTGCAAATCGTTCGTCATACTTGGGTATAGGGGCG
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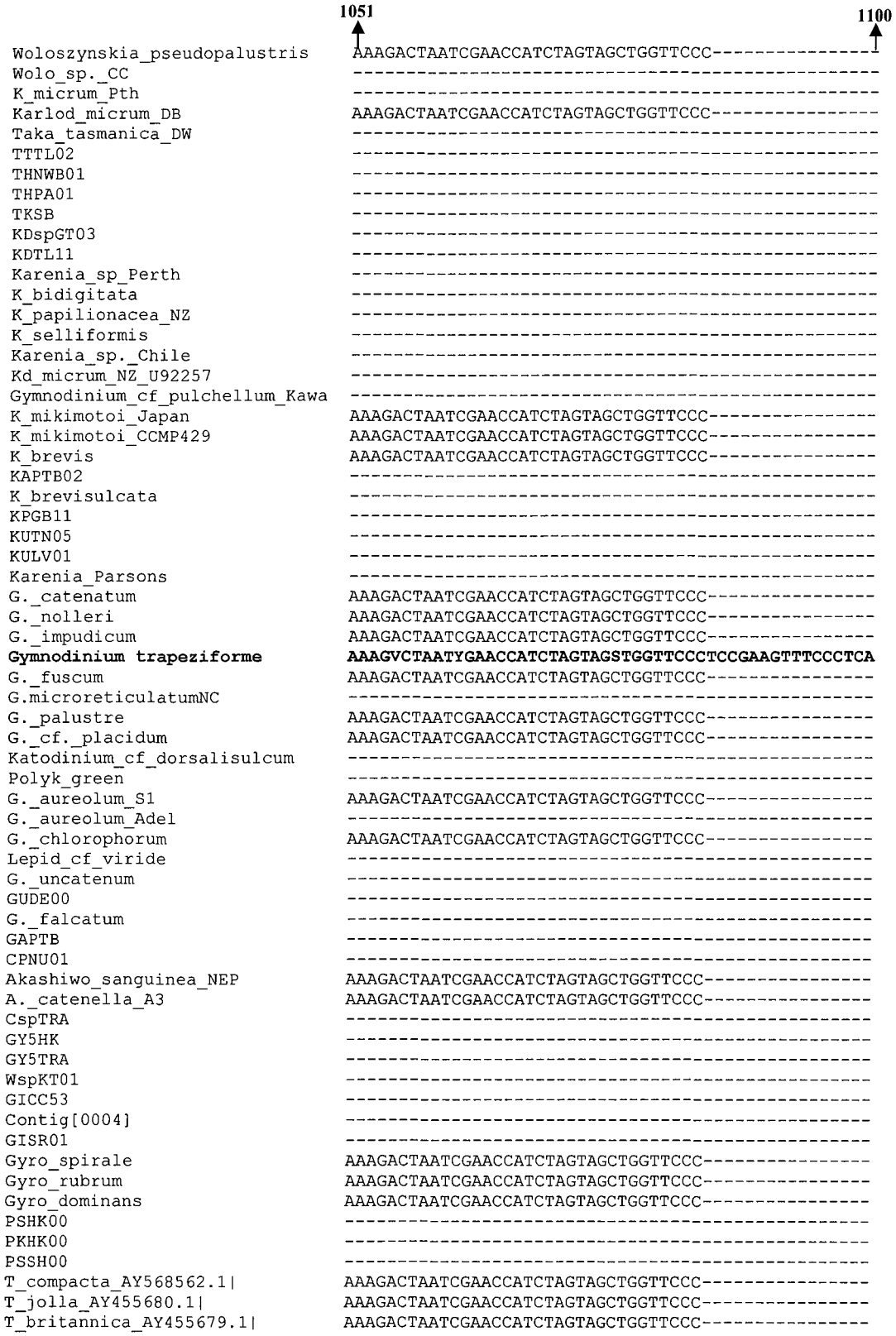
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Wolo_sp._CC ----------
K_micrum_Pth ----------
Karlod_micrum_DB AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
Taka_tasmanica_DW ----------
TTTL02 ----------
THNWB01 ----------
THPA01 ----------
TKSB ----------
KDspGT03 ----------
KDTL11 ----------
Karenia_sp_Perth ----------
K_bidigitata ----------
K_papilionacea_NZ ----------
K_selliformis ----------
Karenia_sp._Chile ----------
Kd_micrum_NZ_U92257 ----------
Gymnodinium_cf_pulchellum_Kawa ----------
K_mikimotoi_Japan AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
K_mikimotoi_CCMP429 AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
K_brevis AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
KAPTB02 ----------
K_brevisulcata ----------
KPGB11 ----------
KUTN05 ----------
KULV01 ----------
Karenia_Parsons ----------
G._catenatum AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
G._nolleri AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
G._impudicum AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
Gymnodinium trapeziforme AAAGVCTAATYGAACCATCTAGTAGSTGGTTCCCTCCGAAGTTTCCCTCA
G._fuscum AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
G.microreticulatumNC ----------
G._palustre AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
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Katodinium_cf_dorsalisulcum ----------
Polyk_green ----------
G._aureolum_S1 AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
G._aureolum_Adel ----------
G._chlorophorum AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
Lepid_cf_viride ----------
G._uncatenum ----------
GUDE00 ----------
G._falcatum ----------
GAPTB ----------
CPNU01 ----------
Akashiwo_sanguinea_NEP AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
A._catenella_A3 AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
CspTRA ----------
GY5HK ----------
GY5TRA ----------
WspKT01 ----------
GICC53 ----------
Contig[0004] ----------
GISR01 ----------
Gyro_spirale AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
Gyro_rubrum AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
Gyro_dominans AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
PSHK00 ----------
PKHK00 ----------
PSSH00 ----------
T_compacta_AY568562.1| AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
T_jolla_AY455680.1| AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------
T_britannica_AY455679.1| AAAGACTAATCGAACCATCTAGTAGCTGGTTCCC----------

	1101 1110
Woloszynskia_pseudopalustris	----------
Wolo_sp._CC	----------
K_micrum_Pth	----------
Karlod_micrum_DB	----------
Taka_tasmanica_DW	----------
TTTL02	----------
THNWB01	----------
THPA01	----------
TKSB	----------
KDspGT03	----------
KDTL11	----------
Karenia_sp_Perth	----------
K_bidigitata	----------
K_papilionacea_NZ	----------
K_selliformis	----------
Karenia_sp._Chile	----------
Kd_micrum_NZ_U92257	----------
Gymnodinium_cf_pulchellum_Kawa	----------
K_mikimotoi_Japan	----------
K_mikimotoi_CCMP429	----------
K_brevis	----------
KAPTB02	----------
K_brevisulcata	----------
KPGB11	----------
KUTN05	----------
KULV01	----------
Karenia_Parsons	----------
G._catenatum	----------
G._nolleri	----------
G._impudicum	----------
Gymnodinium trapeziforme	**GGATAGCTGGAGTT**
G._fuscum	----------
G.microreticulatumNC	----------
G._palustre	----------
G._cf._placidum	----------
Katodinium_cf_dorsalisulcum	----------
Polyk_green	----------
G._aureolum_S1	----------
G._aureolum_Adel	----------
G._chlorophorum	----------
Lepid_cf_viride	----------
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GUDE00	----------
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GY5HK	----------
GY5TRA	----------
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GICC53	----------
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Gyro_rubrum	----------
Gyro_dominans	----------
PSHK00	----------
PKHK00	----------
PSSH00	----------
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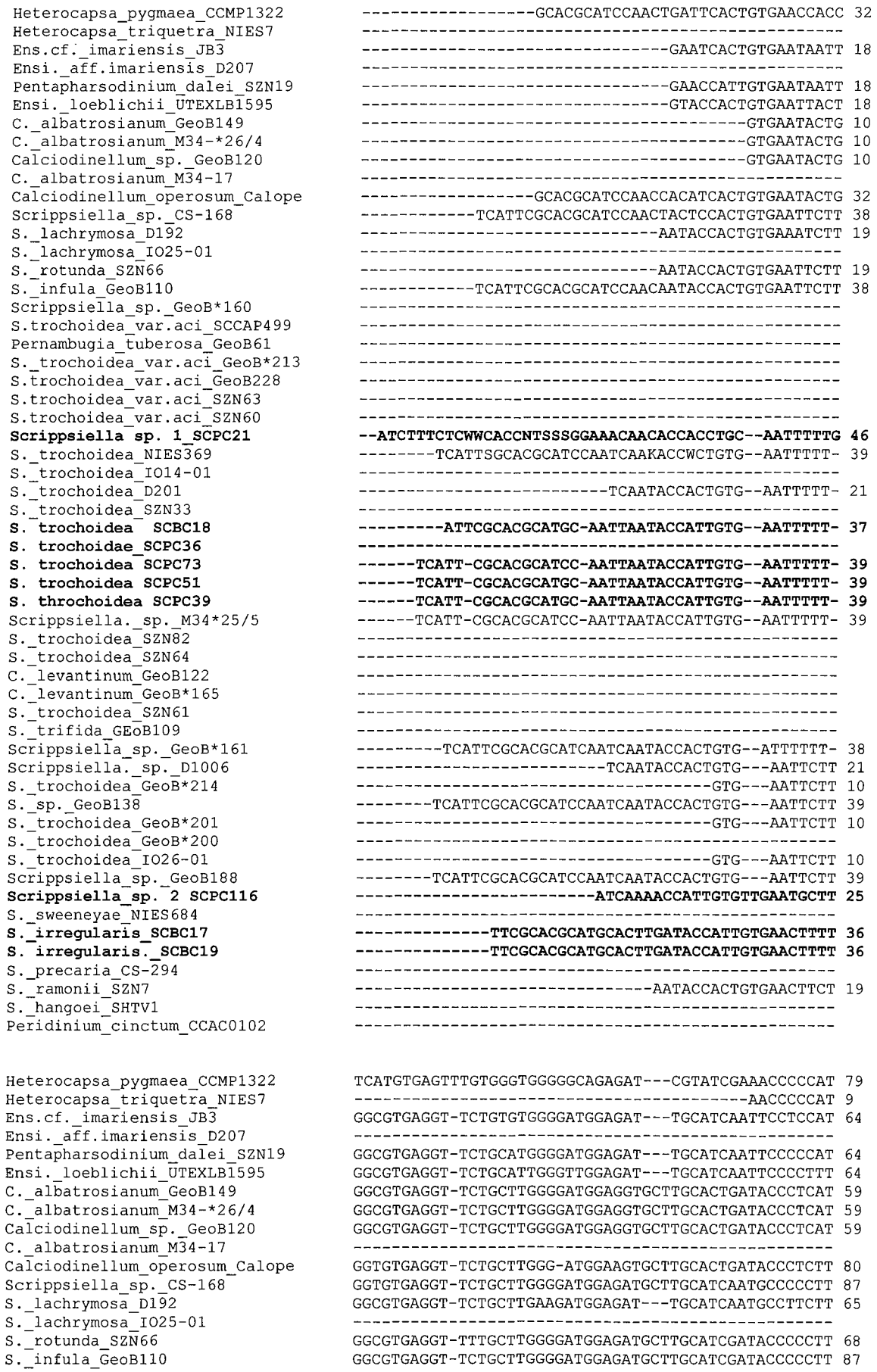
T_jolla_AY455680.1|	----------
T_britannica_AY455679.1|	----------

Figure 2: Sequence alignment of ribosomal RNA of ITS region in Calciodinellaceae



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S.trochoidea_var.aci_SCCAP499      ------------------------------------------------
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S._trochoidea_NIES369              GGCGTGAGGT-TCTGCTTGGGGATGGAGAT-TGCAT--CAATGCCCCCTT 85
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S. trochoidea SCPC36               -------GGT-TTTGTTTGGGGATGGAGAT-TGCAT--CAATGCCCCCTT 39
S. trochoidea SCPC73               GGCGTGAGGT-TTTGTTTGGGGATGGAGAT-TGCAT--CAATGCCCCCTT 85
S. trochoidea .SCPC51              GGCGTGAGGT-TTTGTTTGGGGATGGAGAT-TGCAT--CAATGCCCCCTT 85
S. trochoidea SCPC39               GGCGTGAGGT-TTTGTTTGGGGATGGAGAT-TGCAT--CAATGCCCCCTT 85
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S._trifida_GEoB109                 ----------------------------------CAT--CAATGCCCCCAT 15
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S._precaria_CS-294                 ----------------------------------ATCGATGCCCTCTT 14
S._ramonii_SZN7                    GGTGTGAGGT-TCTGCTTGGGGATGGAGAT---TGCATCGATACCCCCAC 65
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Peridinium_cinctum_CCAC0102        ----------------------------------GCGGCTGTCCCTG 13

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C._albatrosianum_M34-17            ----------AAGGCGGCA-GGG-CTGGAT--GGGTGCTTGT-CAC-C 32
Calciodinellum_operosum_Calope     GCAG-AAGCTCAAAGGCGGCA-GGG-CTGGAT--GGGTGCTTGT-CAC-C 123
Scrippsiella_sp._CS-168            GCAG-AAGCTCAAGGGCGGCA-GGGGCTGGATTCAGGTGCTTGT-CAC-C 133
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S._lachrymosa_IO25-01              ----------GCGACC-GGG-CTGGAT--GGGTGCTTGC-CAC-C 29
S._rotunda_SZN66                   GCAT-CAGCTCAAGGGCGGCA-GGGGCTGGAT--GGGTGCTTGT-CAC-C 112
S._infula_GeoB110                  GCAT-TAGCTCAAGGGCGGCA-GGGGCTGGAT--GGGTGCTTGT-CAC-C 131
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Scrippsiella sp. 1_SCPC21          GTAG-AAGCTCGAAGGCGGCA--GGGCTGGAT--GGGTGCTTGT-CAC-C 138
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S. trochoidea SCPC73               GCAA-AAGCTCAAAGGCGGCA--GGGTTGGAT--GGGTGCTTGT-CAC-C 128
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Scrippsiella._sp._M34*25/5         GCAA-AAGCTCAAAGGCGGCA--GGGTTGGAT--GGGTGCTTGT-CAC-C 128
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Peridinium_cinctum_CCAC0102         GCAG-GGCCTCGTGGGCGATG-CGGTCTGGTA--GGGCGTCGAT-TGC-C 57
                                                *      **   **      *        *

Heterocapsa_pygmaea_CCMP1322        AAGTGTGACTCTCTGTCTCGGCTCCCTGTCCCCGTCGCCA-TTGCTTACC 174
Heterocapsa_triquetra_NIES7         TTGTGCGACTCTCTGGCGTGGCTCCCTGCGCTCGTCGCCG-CTGCTTACC 105
Ens.cf._imariensis_JB3              TCCTTTCTGTTCGCGTCGTCATGT----ACCTTGCATGT--TGATCCG-- 149
Ensi._aff.imariensis_D207           TCCTTTCTGTTCGCGTCGTCATGT----ACCTTGCATGT--TGATCCG-- 81
Pentapharsodinium_dalei_SZN19       TCCTTTCTGTTCGCGTCGTCATGT----ACCTTGCATGT--TGATAT--- 148
Ensi._loeblichii_UTEXLB1595         TCCTTTCTGTTCTTGTCGTCATGT----ACCTTGCCTATT-CCACGTGC- 151
C._albatrosianum_GeoB149            TCCTTTCAGTTCTTGTCGTCACCC----ATTGTTGTTCTT-TGTTTTGCT 147
C._albatrosianum_M34-*26/4          TCCTTTCAGTTCTTGTCGTCACCC----ATTGTTGTTCTT-TGTTTTGCT 147
Calciodinellum_sp._GeoB120          TCCTTTCAGTTCTTGTCGTCACCC----ATTGTTGTTCTT-TGTTTTGCT 147
C._albatrosianum_M34-17             TCCTTTCAGTTCTTGTCGTCACCC----AYTGTTGTTCTT-TGTTTTGCT 77
Calciodinellum_operosum_Calope      TCCTTTCAGTTCTTGTCGTCACCC----ATTGTTGTTCTT-C-CTTTGCT 167
Scrippsiella_sp._CS-168             TCCATTCTGTGCCTGTCGTCACCC----TCCTTTG--CTT-CATTTTGCC 176
S._lachrymosa_D192                  TCCTTTCAGTTCTTGTCGTCACCC----ACCTTTTGCCTT-GATCTTGCC 153
S._lachrymosa_IO25-01               TCCTTTCAGTCTTTGTCGTCACCC----ACCTTTTGCCTT-GATCTTGCC 74
S._rotunda_SZN66                    TCCTTTCAGTTCGTGTCGTCACCC----TCCCTTTGCCTT-AATATAAGT 157
S._infula_GeoB110                   TCCTTTCAGTTTCTGTCGTCACCC----TCCTTTTGCTTT-TATATAT-- 174
Scrippsiella_sp._GeoB*160           TCCTTTCAGTTCTTGTCGTCACTT---CCCTTGTCACTTT----TCTTGC 101
S.trochoidea_var.aci_SCCAP499       TCCTTTCAGTTCCTGTCGTCACTC----ACCCTTTACTTT-T--GCTTGC 81
Pernambugia_tuberosa_GeoB61         TCCTTTCAGTTCTTGTCGTCACCT-----CTTGCTTTTGT-G--TTGCAT 99
S._trochoidea_var.aci_GeoB*213      TCCTTTCAGTGCTTGTCGTCTTCT----TCCTTTCGCCTT-GTATATGTG 103
S.trochoidea_var.aci_GeoB228        TCCTTTCAGTGCTTGTCGTCTTCT----TCCTTTCGCCTT-GTATATGTG 103
S.trochoidea_var.aci_SZN63          TCCTTTCAGTGCTTGTCGTCTTCT----TCCTTTCGCCTT-GTATATGTG 83
S.trochoidea_var.aci_SZN60          TCCTTTCAGTGCTTGTCGTCTTCT----TCCTTTCGCCTT-GTATATGTG 83
Scrippsiella sp. 1 SCPC21           TCCTTTCAGTTCTCGTCGTCACCCA--CTCTTCTTGCTTT-TTTTGTTCT 185
S._trochoidea_NIES369               TCCTTTCAGTTCCTGTCGTCACCT----CCCTTTTGCCTT-T-ATGTTTG 172
S._trochoidea_IO14-01               TCCTTTCAGTTCCTGTCGTCACCT----CCCTTTTGCCTT-T-ATGTTTG 73
S._trochoidea_D201                  TCCTTTCAGTTCCTGTCGCCACCT----CCCTTTTGCCTT-T-ATGTTGG 154
S._trochoidea_SZN33                 TCCTTTCAGTTCCTGTCGCCACCT----CCCTTTTGCCTT-T-ATGTTGG 82
S. trochoidea SCBC18                TCCTTTCAGCTCTTGTCGTCACCT----TCCTTTTGCTTG-T--AATCTT 169
S. trochoidea SCPC36                TCCTTTCAGCTCTTGTCGTCACCT----TCCTTTTGCTTG-T--AATCTT 125
S. trochoidea SCPC73                TCCTTTCAGCTCTTGTCGTCACCT----TCCTTTTGCTTG-T--AATCTT 171
S. trochoidea.SCPC51                TCCTTTCAGCTCTTGTCGTCACCT----TCCTTTTGCTTG-T--AATCTT 171
S. trochoidea SCPC39                TCCTTTCAGCTCTTGTCGTCACCT----TCCTTTTGCTTG-T--AATCTT 171
Scrippsiella._sp._M34*25/5          TCCTTTCAGCTCTTGTCGTCACCT----TCCTTTTGCTTG-T--AATCTT 171
S._trochoidea_SZN82                 TCCTTTCAGCTCTTGTCGTCACCT----TCCTTTTGCTTG-TGAAGTGTT 83
S._trochoidea_SZN64                 TCCTTTCAGCTCTTGTCGTCAACT----TCCTTTTGCTTG-TGAAATGTT 83
C._levantinum_GeoB122               TCCTTTCAGCTCTTGTCGTCAACT----TCCTTTTGCTTG-CGAAGTGTT 74
C._levantinum_GeoB*165              TCCTTTCAGCTCTTGTCGTCAACT----TCCTTTTGCTTG-CGAAGTGTT 73
S._trochoidea_SZN61                 TCCTTTCAGCTCTTGTCGTCACCT----TCCTTTTGCTTG-CGAAGTGCT 83
S._trifida_GEoB109                  TCCTTTCAGTTCCTGTCGTCACCT----TTCTTTTACC-G-TCATGTCTT 102
Scrippsiella_sp._GeoB*161           TCCTTTCAGTTCTTGTCGTCATTT----CCCTTTCGCTTT-TTATGCTTC 172
Scrippsiella._sp._D1006             TCCTTTCAGTTCCTGTCGTCACCC----TCCTTTTGCCTT-T-AT-TTGC 153
S._trochoidea_GeoB*214              TCCTTTCAGTTCCTGTCGTCACCC----TCCTTTTGCCTT-T-AT-TTGC 142
S._sp._GeoB138                      TCCTTTCAGTTCCTGTCGTCACCC----TCCTTTTGCCTT-T-AT-TTGC 171
S._trochoidea_GeoB*201              TCCTTTCAGTTCCTGTCGTCACCT----TTCTTTTGCCTT-T-AT-TTGC 142
S._trochoidea_GeoB*200              TCCTTTCAGTTCCTGTCGTCACCC----TCCTTTTGCCTT-T-AT-TTGC 72
S._trochoidea_IO26-01               TCCTTTCAGTTCCTGTCGTCACCC----TCCTTTTGCCTT-T-AT-TTGC 142
Scrippsiella_sp._GeoB188            TCCTTTCAGTTCCTGTCGTCACCC----TCCTTTTGCCTT-T-AT-TTGC 171
Scrippsiella sp. 2 SCPC116          TCCTTTCAGTTCTTGTCGCCACCT----TCCTTTTGCCTT-T-GTGTTGC 164
S._sweeneyae_NIES684                TCCTTTCAGTTCTTGTCGTCAACT----TCTTTTTGCTTT-T-TTGATGC 83
S. irregularis SCBC17               TCCCTCCAAACCTTGTTGCCACCC----TCTCTCTCGGTT-GAATTCACA 170
S. irregularis SCBC19               TCCCTCCAAACCTTGTTGCCACCC----TCTCTCTCGGTT-GAATTCACA 170
S._precaria_CS-294                  TCCTTTCAATTCCTGTTGGCACCT----TCTCTTT--GCT-TCTTGCTCA 100
S._ramonii_SZN7                     TCCTTTCAGTTCTTGTTGTCATCT----TTCCTCT--GTT-GAATTTGCT 151
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S._hangoei_SHTV1                    TCCTTTCTGTTCTTGTCGTCACTC-----CCTTTTCTACT-TCTTGCTTC 102
Peridinium_cinctum_CCAC0102         CTCTTCCAGGCCTTGTCGCCAAGT------ATCTTGCCTG-CCACACCTC 100
                                                  *

Heterocapsa_pygmaea_CCMP1322        TGTCAAAGGATCTTCCTT--TACATGACTTCTCAA--------GTG---G 211
Heterocapsa_triquetra_NIES7         TGTCTCAGGATCTTGTTCC-TACATTTGTTCCCAA--------GTG---G 143
Ens.cf._imariensis_JB3              ----TTTAAAT---GTCA-------TGTGCTTTGG----AGTGCTT---G 178
Ensi._aff.imariensis_D207           ----TTTAAAT---GTCA-------TGTGCTTTGG----AGTGCTT---G 110
Pentapharsodinium_dalei_SZN19       ----TTTACAT---CTCA-------TGTACTCTGG----AGTGCTT---G 177
Ensi._loeblichii_UTEXLB1595         -------AAGT---GCCA-------TCTTCTCTGG---ACGGGCTG---A 178
C._albatrosianum_GeoB149            C-------TTT---GCAA----GCTTACGTTTCAA-------TGTG---G 173
C._albatrosianum_M34-*26/4          C-------TTT---GCAA----GCTTACGTTTCAA-------TGTG---G 173
Calciodinellum_sp._GeoB120          C-------TTT---GCAA----GCTTACGTTTCAA-------CGTG---G 173
C._albatrosianum_M34-17             C-------TTT---GCAA----GCTTACGTTTCAA-------TGTG---G 103
Calciodinellum_operosum_Calope      T-------TTT---GCAA----ATCTACATTTCAA-------TGTG---G 193
Scrippsiella_sp._CS-168             T-------TCT---GCTC-----GATATACTCCGA-------AGTG---G 201
S._lachrymosa_D192                  T--------TG---GCAA----TCTTACTTGCTGA-------CGTG---G 178
S._lachrymosa_IO25-01               T--------TT---GCAA----TCTTACTTTCTGA-------TGTG---G 99
S._rotunda_SZN66                    TGC-CTCTTGC---GCAGCTTTTTTTACATCCTGA-------AGTG---G 193
S._infula_GeoB110                   ---------------CAG-TTGTTTTACATCCTGAT------AGTG---G 199
Scrippsiella_sp._GeoB*160           T-------TTT---GCAT----TTCTACTTCTTGA-------AATG---G 127
S.trochoidea_var.aci_SCCAP499       T-------TTT---GCAG----CTTTACTTCTTGA-------AATG---G 107
Pernambugia_tuberosa_GeoB61         T-------TTT---GCAG----CCTTACTTCTCAG-------AGTG---G 125
S._trochoidea_var.aci_GeoB*213      C-------TTT---GCAA----C-TTACTTGTTGA-------AATG---G 128
S.trochoidea_var.aci_GeoB228        C-------TTT---GCAA----C-TTACTTGTTGA-------AATG---G 128
S.trochoidea_var.aci_SZN63          C-------TTT---GCAA----C-TTACTTGTTGA-------AATG---G 108
S.trochoidea_var.aci_SZN60          C-------TTT---GCAA----C-TTACTTGTTGA-------AATG---G 108
Scrippsiella sp. 1 SCPC21           C-------TTT---TCTT----C-TTTCGC-TCGA-------AGTG---G 209
S._trochoidea_NIES369               C-------TTT---GCAT----TCTTATCTTTCGA-------AGTG---G 198
S._trochoidea_IO14-01               C-------TTT---GCAT----TCTTATCTTTCGA-------AGTG---G 99
S._trochoidea_D201                  C-------TTT---GCAT----TCTTATATTTTGA-------AATG---G 180
S._trochoidea_SZN33                 C-------TTT---GCAT----TCTTATATTTTGA-------AATG---G 108
S. trochoidea SCBC18                T-------GCT---ACTT----CTTTACCAGTTGA-------AGTG---G 195
S. trochoidea SCPC36                T-------GCT---ACTT----CTTTACCAGTTGA-------AGTG---G 151
S. trochoidea SCPC73                T-------GCT---ACTT----CTTTACCAGTTGA-------AGTG---G 197
S. trochoidea SCPC51                T-------GCT---ACTT----CTTTACCAGTTGA-------AGTG---G 197
S. trochoidea SCPC39                T-------GCT---ACTT----CTTTACCAGTTGA-------AGTG---G 197
Scrippsiella._sp._M34*25/5          T-------GCT---ACTT----CTTTACCAGTTGA-------AGTG---G 197
S._trochoidea_SZN82                 C-------TTT---TCTA----TTTTACCAGTTGA-------AGTG---G 109
S._trochoidea_SZN64                 C-------TTT---GCTA----TTTTACCAGTTGA-------AGTG---G 109
C._levantinum_GeoB122               C-------TTT---GCTA----TGTTACCAGTTGA-------AGTG---G 100
C._levantinum_GeoB*165              C-------TTT---GCTA----TGTTACCAGTTGA-------AGTG---G 99
S._trochoidea_SZN61                 C-------TTT---GCTA----TTTTACCCGTTGA-------AGTG---G 109
S._trifida_GEoB109                  C-------TTT---GCAA----TCTTACCTTTTGA-------AGTG---G 128
Scrippsiella_sp._GeoB*161           C-------GTT---GTGT-----GTAACCCATTGGA------AGTG---G 198
Scrippsiella._sp._D1006             C-------TTT---GCAA----ATTTACTTCT-GA-------AGTG---G 178
S._trochoidea_GeoB*214              C-------TTT---GCAA----ATTTACTTCT-GA-------AGTG---G 167
S._sp._GeoB138                      C-------TTT---GCAA----ATTTACTTCT-GA-------AGTG---G 196
S._trochoidea_GeoB*201              C-------TTT---GCAA----ATTTACTTCT-GA-------AGTG---G 167
S._trochoidea_GeoB*200              C-------TTT---GCAA----ATTTACTTCT-GA-------AGTG---G 97
S._trochoidea_IO26-01               C-------TTT---GCAA----ATTTACTTCT-GA-------AGTG---G 167
Scrippsiella_sp._GeoB188            C-------TTT---GCAA----ATTTACTTCT-GA-------AGTG---G 196
Scrippsiella sp. 2 SCPC116          C-------TTT---GCAG----CTTTACTTCTTGA-------AATG---G 190
S._sweeneyae_NIES684                C-------TTT---GCAT----ACTTACTTTCTGA-------AGTG---G 109
S. irregularis SCBC17               --------TGT---GCAG----CGTTACATCCTTT-------AGCG---G 195
S. irregularis SCBC19               --------TGT---GCAG----CGTTACATCCTTT-------AGCG---G 195
S._precaria_CS-294                  --------ATT---GCAA----TATTACTCTCTGG-------AGCG---G 125
S._ramonii_SZN7                     C-------TAT---GCAG----AATTACGTTCTGT-------AGCG---G 177
S._hangoei_SHTV1                    T-------TGT---CTAA----TACTTCGTTCGGA-------AGTG---G 128
Peridinium_cinctum_CCAC0102         C---------T---GCCC------GTGCTTCTTGAGCATAAGAGTGTGAG 132


Heterocapsa_pygmaea_CCMP1322        A-----TTTCCACTTA----TTCACTTACAACTTTCAGCGACGG-ATGTC 251
Heterocapsa_triquetra_NIES7         G-----CTTCCACTTTAT--TTTACTTACAACTTTCAGCGACGG-ATGTC 185
Ens.cf._imariensis_JB3              ----GTCACTTCTTTT----CTTTTTTACAACTTTCAGCGATCG-ATGTC 219
Ensi._aff.imariensis_D207           ----GTCACTTCTTTT----CTTTTTTACAACTTTCAGCGATGG-ATGTC 151
Pentapharsodinium_dalei_SZN19       ----GTCACTTCTTTT----CTTCCTTACAACTTTCAGCGATCG-ATGTC 218
Ensi._loeblichii_UTEXLB1595         ------CGTCCGCATT----TTCTCTTACAACTTTCAGTGATCG-ATGTC 217
C._albatrosianum_GeoB149            C-----TGTCCACCTT----GAA--TCACAACTTTCAGCGATCG-ATGTC 211
C._albatrosianum_M34-*26/4          C-----TGTCCACCTT----GAA--TCACAACTTTCAGCGATCG-ATGTC 211
Calciodinellum_sp._GeoB120          C-----TGTCCACCTT----GAA--TCACAACTTTCAGCGATCG-ATGTC 211
C._albatrosianum_M34-17             C-----TGTCCACCTT----GAA--TCACAACTTTCAGCGATCG-ATGTC 141
Calciodinellum_operosum_Calope      C-----TGTCCACTTT----GAA--TTACAACTTTCAGCGATCG-ATGTC 231
Scrippsiella_sp._CS-168             T-----TGTCCACTTT----CTTTCTTACAACTTTCAGCGATCG-ATGTC 241
S._lachrymosa_D192                  T-----TGTCCATTTC----CTT-CTTACAACTTTCAGTGATCG-ATGTC 217
S._lachrymosa_IO25-01               T-----TGTCCATTTC----CTT-CTTACAACTTTCAGTGATCG-ATGTC 138
S._rotunda_SZN66                    T---TGTGTCCACTTT----CTT-CTTACAACTTTCAGCGATCG-ATGTC 234
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S._infula_GeoB110                    TCATTATGTCCACTTT----CTTTCTTACAACTTTCAGCGATCG-ATGTC 244
Scrippsiella_sp._GeoB*160            G----TTTCCCATTTC----TTTTCTTACAACTTTCAGCGATCG-ATGTC 168
S.trochoidea_var.aci_SCCAP499        -----TTATCCATGTC----TTCT-TTACAACTTTCAGCGATCG-ATGTC 146
Pernambugia_tuberosa_GeoB61          -----TCTTCCACTTT----CTTT-TTACAACTTTCAGCGATCG-ATGTC 164
S._trochoidea_var.aci_GeoB*213       -----TTGTCCATTTC----CCTC-TTACAACTTTCAGCGATCG-ATGTC 167
S.trochoidea_var.aci_GeoB228         -----TTGTCCATTTC----CCTC-TTACAACTTTCAGCGATCG-ATGTC 167
S.trochoidea_var.aci_SZN63           -----TTGTCCATTTC----CCTC-TTACAACTTTCAGCGATCG-ATGTC 147
S.trochoidea_var.aci_SZN60           -----TTGTCCATTTC----CCTC-TTACAACTTTCAGCGATCG-ATGTC 147
Scrippsiella sp. 1 SCPC21            -----TTCTCCACTTC----ACTC-TTACAACTTTCAGCGATCG-ATGTC 248
S._trochoidea_NIES369                -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 237
S._trochoidea_IO14-01                -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 138
S._trochoidea_D201                   -----TTGTCCACTTT----CTTA-T-ACAACTTTCAGCGATCG-ATGTC 218
S._trochoidea_SZN33                  -----TTGTCCACTTT----CTTA-T-ACAACTTTCAGCGATCG-ATGTC 146
S. trochoidea SCBC18                 -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 234
S. trochoidea SCPC36                 -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATGC-ATGTC 190
S. trochoidea SCPC73                 -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATAG-ATGTC 236
S. trochoidea SCPC51                 -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 236
S. trochoidea SCPC39                 -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 236
Scrippsiella._sp._M34*25/5           -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 236
S._trochoidea_SZN82                  -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 148
S._trochoidea_SZN64                  -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 148
C._levantinum_GeoB122                -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATTG-ATGTC 139
C._levantinum_GeoB*165               -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATTG-ATGTC 138
S._trochoidea_SZN61                  -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 148
S._trifida_GEoB109                   -----TTCTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 167
Scrippsiella_sp._GeoB*161            -----TTCTCCGCTTT----CTCT-TTACAACTTTCAGCGATCG-ATGTC 237
Scrippsiella._sp._D1006              -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 217
S._trochoidea_GeoB*214               -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 206
S._sp._GeoB138                       -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 235
S._trochoidea_GeoB*201               -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 206
S._trochoidea_GeoB*200               -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 136
S._trochoidea_IO26-01                -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 206
Scrippsiella_sp._GeoB188             -----TTGTCCACTTT----CTTC-TTACAACTTTCAGCGATCG-ATGTC 235
Scrippsiella_ sp. 2 SCPC116          -----TTGTCCATTTC----CTTC-TTACAACTTTCAGCGATTG-ATGTC 229
S._sweeneyae_NIES684                 -----TTCTCCACTTG----CTTC-TTACAACTTTCAGCGATCG-ATGTC 148
S. irregularis SCBC17                -----TTCTCCGCTCC----AACC-TTTTGACTTTCAGTGATCG-ATGTC 234
S. irregularis SCBC19                -----TTCTCCGCTCC----AACC-TTTTGACTTTCAGTGATCG-ATGTC 234
S._precaria_CS-294                   -----TTGTCTGCTCT----CGTC-TTTTGACTTTCAGCGATCG-ATGTC 164
S._ramonii_SZN7                      -----TCTTCCGCGAT----CCTC-CTTTGACTTTCAGCGATCG-ATGTC 216
S._hangoei_SHTV1                     TTTCCCACTTCTGCCT----CTTT-TTACAACTTTCAGCGATCG-ATGTC 172
Peridinium_cinctum_CCAC0102          CCTCGCTTCACAYTCACASACACACCCACCACATTCAGTGACGG-ATGTC 181
                                                          ** ***** **    *****

Heterocapsa_pygmaea_CCMP1322         TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 300
Heterocapsa_triquetra_NIES7          TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 234
Ens.cf._imariensis_JB3               TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 268
Ensi._aff.imariensis_D207            TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 200
Pentapharsodinium_dalei_SZN19        TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 267
Ensi._loeblichii_UTEXLB1595          TCGGCTCGAGCAATGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 266
C._albatrosianum_GeoB149             TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 260
C._albatrosianum_M34-*26/4           TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 260
Calciodinellum_sp._GeoB120           TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 260
C._albatrosianum_M34-17              TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 190
Calciodinellum_operosum_Calope       TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 280
Scrippsiella_sp._CS-168              TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 290
S._lachrymosa_D192                   TCGGCTCGAGCAATGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 266
S._lachrymosa_IO25-01                TCGGCTCGAGCAATGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 187
S._rotunda_SZN66                     TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 283
S._infula_GeoB110                    TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 293
Scrippsiella_sp._GeoB*160            TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 217
S.trochoidea_var.aci_SCCAP499        TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 195
Pernambugia_tuberosa_GeoB61          TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 213
S._trochoidea_var.aci_GeoB*213       TCGGCTCGAACAACGATG-AAGGGCGTAGCGAAGTGTGATAAGCATTGTG 216
S.trochoidea_var.aci_GeoB228         TCGGCTCGAACAACGATG-AAGGGCGTAGCGAAGTGTGATAAGCATTGTG 216
S.trochoidea_var.aci_SZN63           TCGGCTCGAACAACGATG-AAGGGCGTAGCGAAGTGTGATAAGCATTGTG 196
S.trochoidea_var.aci_SZN60           TCGGCTCGAACAACGATG-AAGGGCGTAGCGAAGTGTGATAAGCATTGTG 196
Scrippsiella sp. 1 SCPC21            TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 297
S._trochoidea_NIES369                TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 286
S._trochoidea_IO14-01                TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 187
S._trochoidea_D201                   TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 267
S._trochoidea_SZN33                  TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 195
S. trochoidea SCBC18                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 283
S. trochoidea SCPC36                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 239
S. trochoidea SCPC73                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 285
S. trochoidea SCPC51                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 285
S. trochoidea SCPC39                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 285
Scrippsiella._sp._M34*25/5           TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 285
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S._trochoidea_SZN82                    TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 197
S._trochoidea_SZN64                    TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 197
C._levantinum_GeoB122                  TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 188
C._levantinum_GeoB*165                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 187
S._trochoidea_SZN61                    TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 197
S._trifida_GEoB109                     TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 216
Scrippsiella_sp._GeoB*161              TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 286
Scrippsiella._sp._D1006                TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 266
S._trochoidea_GeoB*214                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 255
S._sp._GeoB138                         TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 284
S._trochoidea_GeoB*201                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 255
S._trochoidea_GeoB*200                 TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 185
S._trochoidea_IO26-01                  TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 255
Scrippsiella_sp._GeoB188               TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 284
Scrippsiella sp.2 SCPC116              TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGCGATAAGCATTGTG 278
S._sweeneyae_NIES684                   TCGGCTCGAACAACGATG-AAGGGCGCAGCGAAGTGTGATAAGCATTGTG 197
S. irregularis SCBC17                  TCGGCTCGAACAACGATG-AAGGGCACAGCAAAGTGTGATAAGCATTGTG 283
S. irregularis SCBC19                  TCGGCTCGAACAACGATG-AAGGGCACAGCAAAGTGTGATAAGCATTGTG 283
S._precaria_CS-294                     TCGGCTCGAACAACGATG-AAGGGCACAGCAAAGTGTGATAAGCATTGTG 213
S._ramonii_SZN7                        TCGGCTCGAACAACGATG-AAGGGCACAGCAAAGTGTGATAAGCATTGTG 265
S._hangoei_SHTV1                       TCGGCTCGAACAACGATG-AAGGGCGCAGCGAACTGTGATAAGCATTGTG 221
Peridinium_cinctum_CCAC0102            TTGGCTTGAGCAGCGATG-AAGGGCGCAGCGAACTGCGATACGCATTGTG 230
                                       * **** ** **  **** ******  *** ** ** **** ********

Heterocapsa_pygmaea_CCMP1322           AATTGCAGAATTCCGTGAACCAATAGGGATTTGAACGTACACTGCGCTTT 350
Heterocapsa_triquetra_NIES7            AATTGCAGAATTCCGTGAACCAATAGGGATTTGAACGTACACTGCGCTTT 284
Ens.cf._imariensis_JB3                 AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 318
Ensi._aff.imariensis_D207              AATTGCAGAATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 250
Pentapharsodinium_dalei_SZN19          AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 317
Ensi._loeblichii_UTEXLB1595            AATTGCAGGATTCCGTCAGCCAATAGGGACTTGAACGTACACTGTGCTTT 316
C._albatrosianum_GeoB149               AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 310
C._albatrosianum_M34-*26/4             AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 310
Calciodinellum_sp._GeoB120             AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 310
C._albatrosianum_M34-17                AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 240
Calciodinellum_operosum_Calope         AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGTGCTTT 330
Scrippsiella_sp._CS-168                AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 340
S._lachrymosa_D192                     AATTGCAGGATTCCGTGAGCCAATAGGGACTTGAACGTACACTGTGCTTT 316
S._lachrymosa_IO25-01                  AATTGCAGGATTCCGTGAGCCAATAGGGACTTGAACGTACACTGTGCTTT 237
S._rotunda_SZN66                       AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 333
S._infula_GeoB110                      AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 343
Scrippsiella_sp._GeoB*160              AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 267
S.trochoidea_var.aci_SCCAP499          AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 245
Pernambugia_tuberosa_GeoB61            AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 263
S._trochoidea_var.aci_GeoB*213         AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 266
S.trochoidea_var.aci_GeoB228           AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 266
S.trochoidea_var.aci_SZN63             AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 246
S.trochoidea_var.aci_SZN60             AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 246
Scrippsiella sp. 1 SCPC21              AATTGCAGGATTCCGTGAACCAATAGAGACTTGAACGTACACTGCGCTTT 347
S._trochoidea_NIES369                  AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACASTGCGCTTT 336
S._trochoidea_IO14-01                  AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 237
S._trochoidea_D201                     AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 317
S._trochoidea_SZN33                    AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 245
Scrippsiella_sp_SCBC18                 AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 333
Scrippsiella_sp._SCPC36                AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 289
scrippsiella_sp._SCPC73                AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 335
Scrippsiella._sp.SCPC51                AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 335
Scrippsiella_sp._SCPC39                AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 335
Scrippsiella._sp._M34*25/5             AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 335
S._trochoidea_SZN82                    AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 247
S._trochoidea_SZN64                    AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 247
C._levantinum_GeoB122                  AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 238
C._levantinum_GeoB*165                 AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 237
S._trochoidea_SZN61                    AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 247
S._trifida_GEoB109                     AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 266
Scrippsiella_sp._GeoB*161              AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 336
Scrippsiella._sp._D1006                AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 316
S._trochoidea_GeoB*214                 AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 305
S._sp._GeoB138                         AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 334
S._trochoidea_GeoB*201                 AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 305
S._trochoidea_GeoB*200                 AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 235
S._trochoidea_IO26-01                  AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 305
Scrippsiella_sp._GeoB188               AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 334
Scrippsiella sp.2 SCPC116              AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 328
S._sweeneyae_NIES684                   AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 247
S. irregularis SCBC17                  AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 333
S. irregularis SCBC19                  AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 333
S._precaria_CS-294                     AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 263
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S._ramonii_SZN7                          AATTGCAGGATTCCGTGAACCAATAGGGACTTGAACGTACACTGCGCTTT 315
S._hangoei_SHTV1                         AATTGCAGAATTCCGTGAACCAATAGGGACTTGAACGTAAACTGCGCTTT 271
Peridinium_cinctum_CCAC0102              AATTGCAGAATTCTGTGAATCAATAGAGACTTGAATGCGCACTGCGCTTT 280
                                         ******** **** ** *  ****** ** ***** *   * ** *****

Heterocapsa_pygmaea_CCMP1322             CGGGACATCCCTGAAAGCATGCTTGCTTGAGTGTCTACTCCATCTAATGC 400
Heterocapsa_triquetra_NIES7              CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCTACTCCATCTAATGC 334
Ens.cf._imariensis_JB3                   CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-CCATCTTCTGC 367
Ensi._aff.imariensis_D207                CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCTATTCCATCTTCTGC 300
Pentapharsodinium_dalei_SZN19            CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-CCATCTTCTGC 366
Ensi._loeblichii_UTEXLB1595              CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAG-CCACCTACTGC 365
C._albatrosianum_GeoB149                 CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCAAT-TCTTTTCATTC 359
C._albatrosianum_M34-*26/4               CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCAAT-TCTTTTCATTC 359
Calciodinellum_sp._GeoB120               CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCAAT-TCTTTTCATTC 359
C._albatrosianum_M34-17                  CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCAAT-TCTTTTCATTC 289
Calciodinellum_operosum_Calope           CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCAAT-TCCTTTCCTTC 379
Scrippsiella_sp._CS-168                  CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAA-TCTCTTTATTC 389
S._lachrymosa_D192                       CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-GTCATTCATTC 365
S._lachrymosa_IO25-01                    CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-GTCATTCATTC 286
S._rotunda_SZN66                         CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-TCCTTTCATTC 382
S._infula_GeoB110                        CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-TCCTTTTATTC 392
Scrippsiella_sp._GeoB*160                CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-T-CTTTTATTC 315
S.trochoidea_var.aci_SCCAP499            CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-T-TCTTTATTC 293
Pernambugia_tuberosa_GeoB61              CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-TACCTTTATTC 312
S._trochoidea_var.aci_GeoB*213           CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTCCCTTATTC 315
S.trochoidea_var.aci_GeoB228             CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTCCCTTATTC 315
S.trochoidea_var.aci_SZN63               CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTCCCTTATTC 295
S.trochoidea_var.aci_SZN60               CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTCCCTTATTC 295
Scrippsiella sp. 1 SCPC21                CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCGTC-TGCTCTTATTC 396
S._trochoidea_NIES369                    CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTCCTTTATTC 385
S._trochoidea_IO14-01                    CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTCCTTTATTC 286
S._trochoidea_D201                       CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTCCTTTATTC 366
S._trochoidea_SZN33                      CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCGAC-TTCCTTTATTC 294
S. trochoidea SCBC18                     CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTATTTCTTC 382
S. trochoidea SCPC36                     CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTATTTCTTC 338
S. trochoidea SCPC73                     CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTATTTCTTC 384
S. trochoidea SCPC51                     CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTATTTCTTC 384
S. trochoidea SCPC39                     CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTATTTCTTC 384
Scrippsiella._sp._M34*25/5               CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTATTTCTTC 384
S._trochoidea_SZN82                      CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTATTTCTTC 296
S._trochoidea_SZN64                      CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTCTTC 296
C._levantinum_GeoB122                    CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTACTTCTTC 287
C._levantinum_GeoB*165                   CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTACTTCTTC 286
S._trochoidea_SZN61                      CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTATTTATTC 296
S._trifida_GEoB109                       CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTATTC 315
Scrippsiella_sp._GeoB*161                TGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-TTTCCTAATTC 385
Scrippsiella._sp._D1006                  CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTATTC 365
S._trochoidea_GeoB*214                   CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTATTC 354
S._sp._GeoB138                           CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTATTC 383
S._trochoidea_GeoB*201                   CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTATTC 354
S._trochoidea_GeoB*200                   CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTATTC 284
S._trochoidea_IO26-01                    CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTATTC 354
Scrippsiella_sp._GeoB188                 CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAC-TTTCTTTATTC 383
Scrippsiella sp.2 SCPC116                CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCAAT-ATCCTTTATTC 377
S._sweeneyae_NIES684                     CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAT-TTCATTTATTC 296
S. irregularis SCBC17                    CGGGATATCCCTGAAAGCATGCTTGCTTCAGTGTCCAA-T-CCTTTCTTC 381
S. irregularis SCBC19                    CGGGATATCCCTGAAAGCATGCTTGCTTCAGTGTCCAA-T-CCTTTCTTC 381
S._precaria_CS-294                       CGGGATATCCCTGAAAGCATGCCTGCTTCAGCGTCCAT-T-CGCTGATTC 311
S._ramonii_SZN7                          CGGGATATCCCTGAAAGCATGCTTGCTTCAGCGTCCAT-T-CTCTGCTTC 363
S._hangoei_SHTV1                         CGGGATATCCCTGAAAGCATGCCTGCTTCAGTGTCCAATCCATTTGATTC 321
Peridinium_cinctum_CCAC0102              TGGGACATCCCCAAAAGCAGGCCTGCTTCAGTGTCCGT-GCTGTCTGGCA 329
                                          **** *****  ****** ** ***** ** ***

Heterocapsa_pygmaea_CCMP1322             CAGTGGCTAAATCCTCC----CTG----CGGGGGTTGGCCACTTGTGCGT 442
Heterocapsa_triquetra_NIES7              CAGCCTCTAAATCCTCC----TTG----CGAGGGTTGGTGGCCTGCGCGT 376
Ens.cf._imariensis_JB3                   CAGTAATGT-CTTCCAC----CTGAT-GTGG-TGCAGTTG-CTTGTGTGT 409
Ensi._aff.imariensis_D207                CAGTAATGT-CTTCCAC----CTGAT-GTGG-TGCAGTTG-CTTGTGTGT 342
Pentapharsodinium_dalei_SZN19            CAGCGACGT-CTTCCAC----CTCGT-GTGG-TGCAGTTG-CTTGCGCGT 408
Ensi._loeblichii_UTEXLB1595              CAGTGACTA-CTTCCAC----------ATAGGTTTGGACCAGTCTTCCGT 404
C._albatrosianum_GeoB149                 TAGCAACAT-CTTCCAC---TTCGG---TGG-TCCTGTTG-CTTCAGAGC 400
C._albatrosianum_M34-*26/4               TAGCAACAT-CTTCCAC---TTCGG---TGG-TCCTGTTG-CTTCAGAGC 400
Calciodinellum_sp._GeoB120               TAGCAACAT-CTTCCAC---TTCGG---TGG-TCCTGTTG-CTTCAGAGC 400
C._albatrosianum_M34-17                  TAGCAACAT-CTTCCAC---TTCGG---TGG-TCCTGTTG-CTTCAGAGC 330
Calciodinellum_operosum_Calope           TAGCAACAT-CTTCCAC---TTCGG---TGG-TCCTGTTG-CCTCAGAGC 420
Scrippsiella_sp._CS-168                  TGGCAACAC-CTTCCACCCTTGTGG---TGG-TACCGTTG-CTTCAGTGT 433
S._lachrymosa_D192                       CGGCAACATGCCCCCAT---TATTG---TGG-TACCGTTG-CCTGTGTGT 407
S._lachrymosa_IO25-01                    CGGCAACATGCCCCCAT---TATTG---TGG-TACCGTTG-CCTGCGTGT 328
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S._rotunda_SZN66                    TGGCAACAC-CTCCCACATTTCTG----TGG-CACCGTTG-CTTCAGTGT 425
S._infula_GeoB110                   TGGCAACAC-CTCCCACCGCTCTGG---TGG-CACCGTTG-CTTCAGTGT 436
Scrippsiella_sp._GeoB*160           CAGCAACAC-CTTCCAT--TCCGG----TGG-TACTGTTG-CTTGGGTGT 356
S.trochoidea_var.aci_SCCAP499       CAGCAACAC-CTTCCAC--TTCTGCTG-TGG-TACTGTTG-CTTGGGTGT 337
Pernambugia_tuberosa_GeoB61         CAGCAACAC-CTTCCAC--TTTCG----TGG-TCTTGTTG-CTTTGGTGT 353
S._trochoidea_var.aci_GeoB*213      CTGCAACAA-CTTCCAC--ATTTG----TGG-TACTGTTG-CTTGGGTGT 356
S.trochoidea_var.aci_GeoB228        CTGCAACAA-CTTCCAC--ATTTG----TGG-TACTGTTG-CTTGGGTGT 356
S.trochoidea_var.aci_SZN63          CTGCAACAA-CTTCCAC--ATTTG----TGG-TACTGTTG-CTTGGGTGT 336
S.trochoidea_var.aci_SZN60          CTGCAACAA-CTTCCAC--ATTTG----TGG-TACTGTTG-CTTGGGTGT 336
Scrippsiella sp. 1 SCPC21           CGGCAACTG-CTGCTGC---TTCGG---TGGCTATTGTTG-CTCGGGTGT 438
S._trochoidea_NIES369               CAGCAACAC-CTTCCAC--TTTTG----TGG-TACCGTTG-CTTGGGTGT 426
S._trochoidea_IO14-01               CAGCAACAC-CTTCCAC--TTTTG----TGG-TACCGTTG-CTTGGGTGT 327
S._trochoidea_D201                  CAGCAACAC-CTTCCAC--TTTTG----TGG-TACCGTTG-CTTGGGTGT 407
S._trochoidea_SZN33                 CAGCAACAC-CTTCCAC--TTTTG----TGG-TACCGTTG-CTTGGGTGT 335
S. trochoidea SCBC18                CAGCAGCAT-CTTCCAC--TTTTG----TGG-TACTGTTG-CTTGGGTGT 423
S. trochoidea SCPC36                CAGCAGCAT-CTTCCAC--TTTTG----TGG-TACTGTTG-CTTGGGTGT 379
S. trochoidea SCPC73                CAGCAGCAT-CTTCCAC--TTTTG----TGG-TACTGTTG-CTTGGGTGT 425
S. trochoidea SCPC51                CAGCAGCAT-CTTCCAC--TTTTG----TGG-TACTGTTG-CTTGGGTGT 425
S. trochoidea SCPC39                CAGCAGCAT-CTTCCAC--TTTTG----TGG-TACTGTTG-CTTGGGTGT 425
Scrippsiella._sp._M34*25/5          CAGCAGCAT-CTTCCAC--ATTTG----TGG-TACTGTTG-CTTGGGTGT 425
S._trochoidea_SZN82                 CAGCAACAT-CTTCCAC--TTTTG----TGG-TATTGTTG-CTTGGGTGT 337
S._trochoidea_SZN64                 CAGCAACAT-CTTCCAC--TTTTG----TGG-TACTGTTG-CTTGGGTGT 337
C._levantinum_GeoB122               CAGCAGCAT-CTTCCACTGTTGTG----TGG-TATTGTTG-CTTGGATGT 330
C._levantinum_GeoB*165              CAGCAGCAT-CTTCCACTGTTGTG----TGG-TATTGTTG-CTTGGATGT 329
S._trochoidea_SZN61                 CAGCAGCAT-CTTCCAC--TTGTG----TGG-TCTTGTTG-CTTGGATGT 337
S._trifida_GEoB109                  CAGCAACAC-CTTCCAC--TTTTG----TGG-CCCTGTTG-CTTGGGTGT 356
Scrippsiella_sp._GeoB*161           CAGCAACAC-CTTCCAC--CTTTG----TGG-TAATGTTG-CTTGGGTGT 426
Scrippsiella._sp._D1006             CGGCAACAC-CTTCCAC--TTTCG----TGG-TACCGTTG-CTTGAGTGT 406
S._trochoidea_GeoB*214              CGGCAACAC-CTTCCAC--TTTCG----TGG-TACCGTTG-CTTGAGTGT 395
S._sp._GeoB138                      CGGCAACAC-CTTCCAC--TTTCG----TGG-TACCGTTG-CTTGAGTGT 424
S._trochoidea_GeoB*201              CGGCAACAC-CTTCCAC--TTTTG----TGG-TCCTGTTG-CTTGAGTGT 395
S._trochoidea_GeoB*200              CGGCAACAC-CTTCCAC--TTTTG----TGG-TACTGTTG-CTCGAGTGT 325
S._trochoidea_IO26-01               CGGCAACAC-CTTCCAC--TTTTG----TGG-TACTGTTG-CTCGAGTGT 395
Scrippsiella_sp._GeoB188            CGGCAACAC-CTTCCAC--TTTTG----TGG-TACTGTTG-CTCGAGTGT 424
Scrippsiella sp.2 SCPC116           CGGCAACTT-CTTCCAC--TCTTG----TGG-TGCTGTTG-CTTGGGTGT 418
S._sweeneyae_NIES684                CCGCAACAC-CTTCCAC--TTTTG----TGG-TAACGTTG-CTTGGGAGT 337
S. irregularis SCBC17               CCACAACCC-CAGCCCT-----TGC---TGG-TAGCGTTG-TCTGGGTGT 420
S. irregularis SCBC19               CCACAACCC-CAGCCCT-----TGC---TGG-TAGCGTTG-TCTGGGTGT 420
S._precaria_CS-294                  CCACAACCC-CACCTCC----TTG----TGG-TAGCGTTG-TATGGGTGT 350
S._ramonii_SZN7                     CCGCAACCC--ACCCAC-----CGG---TGG-TAGTGTTGCTTTGGGTGT 402
S._hangoei_SHTV1                    CGGCGACAT-AATCCAC--ATCTG----TGG-TGTTGTTG-TCTGAGTGT 362
Peridinium_cinctum_CCAC0102         TGGCAGCGAAGTCACGC-----CGA---TGGAGACCAGCTGTTGGCATGT 371
                                                                                    *

Heterocapsa_pygmaea_CCMP1322        ATCTGTGTGTTAGGGCGCAA-CTTGTTGCCCCTGACGC-ATTCAATGTAT 490
Heterocapsa_triquetra_NIES7         GTCTGTGTGTTAGGGCGTGGGTTTCCCACCCCTGACGC-ATTCAATGCAT 425
Ens.cf._imariensis_JB3              GGTTGTGCGTTAAGGAGC---TGTGCTGCCCTTGACGC-ATGCAGTGCAT 455
Ensi._aff.imariensis_D207           GGTTGTGCGTTAAGGAGC---TGTGCTGCCCTTGACGC-ATGCAGTGCAT 388
Pentapharsodinium_dalei_SZN19       GCTTGTGCGTTAAGGAGC---TCTGCTGCCCCTGACGC-ATTTAGTGCAT 454
Ensi._loeblichii_UTEXLB1595         ACCTGCGCGTCAAGGAGC-----TG-TGCCCCTGACGC-GTTTAAAGCAT 447
C._albatrosianum_GeoB149            TTTTGTGCATTAGAGTGC---CTTGYTGCCTCTGATGT-GCTTAATGGTT 446
C._albatrosianum_M34-*26/4          TTTTGTGCATTAGAGTGC---CTTGYTGCCTCTGATGT-GCTTAATGGTT 446
Calciodinellum_sp._GeoB120          TTTTGTGCATTAGAGTGC---CTTCTTGCCTCTGATGT-GCTTAATGGTT 446
C._albatrosianum_M34-17             TTTTGTGCATTAGAGTGC---CTTGCTGCCTCTGATGT-GCTTAATGGTT 376
Calciodinellum_operosum_Calope      TTTTGTGCATTAGAGTGC---CTTGTTGCCTCTGATGT-GCTTAATACTT 466
Scrippsiella_sp._CS-168             GTTTGTGTGTTAGAGAGC---CTTGTTGCCTCTGATGC-GCTTAGTGCAT 479
S._lachrymosa_D192                  GCTTGTGTGTTAGAGTGC---TTTGCCGCCTCTGACGC-GCTTAACGCAT 453
S._lachrymosa_IO25-01               GCTTGTGCCTTAGGGTGC---TTTGCCGCCTCTGACGC-GCTTAACGCAT 374
S._rotunda_SZN66                    GTTTGTGCGTTAGAGTGC---TTTGCGGCCTCTGACGC-GCTAAATTCAT 471
S._infula_GeoB110                   GTTTGTGCGTTAGAGTGC---CTTGCGGCCTCTGACGC-GCTAAATCCAT 482
Scrippsiella_sp._GeoB*160           GTTTGTGCGTTAGAGCGC---CTTGCTGCCTCTGACGT-GCTTAATGTAT 402
S.trochoidea_var.aci_SCCAP499       GCTTGTGCGTTAGAGCGC---CTTGTTGCCTCTGATGC-GCTCAATGCAT 383
Pernambugia_tuberosa_GeoB61         GCTTGTGCGTTAGAGAGC---CCTGCTGCCTCTGACGC-ATTCAACTCAT 399
S._trochoidea_var.aci_GeoB*213      GCTTGTGTGTTAAAGTGC---CCTGCTGCCTTTGACGC-ATTTGGTGCAT 402
S.trochoidea_var.aci_GeoB228        GCTTGTGTGTTAAAGTGC---CCTGCTGCCTTTGACGC-ATTTGGTGCAT 402
S.trochoidea_var.aci_SZN63          GCTTGTGTGTTAAAGTGC---CCTGCTGCCTTTGACGC-ATTTGGTGCAT 382
S.trochoidea_var.aci_SZN60          GCTTGTGTGTTAAAGTGC---CCTGCTGCCTTTGACGC-ATTTGGTGCAT 382
Scrippsiella sp. 1 SCPC21           GCTTGTGTGTCAAAGTGC---CCTGCTGCCTTTGACGC-ATTTAAGTCAT 484
S._trochoidea_NIES369               GCTTGTGCGTCAAAGTGC---CTTGCTGCCTCTGACGC-GTTCAATGCAT 472
S._trochoidea_IO14-01               GCTTGTGCGTCAAAGTGC---CTTGCTGCCTCTGACGC-GTTCAATGCAT 373
S._trochoidea_D201                  GCTTGTGTGTCAAAGTGC---TTTGCTGCCTCTGACGC-GTTCAATGCAT 453
S._trochoidea_SZN33                 GCTTGTGTGTCAAAGTGC---TTTGCTGCCTCTGACGC-GTTCAATGCAT 381
S. trochoidea SCBC18                GCTTGTGCGTCAAAGTGC---TTTGCTGCCTTTGACGT-GTTCAATGCAT 469
S. trochoidea SCPC36                GCTTGTGCGTCAAAGTGC---TTTGCTGCCTTTGACGT-GTTCAATGCAT 425
S. trochoidea SCPC73                GCTTGTGCGTCAAAGTGC---TTTGCTGCCTTTGACGT-GTTCAATGCAT 471
S. trochoidea SCPC51                GCTTGTGCGTCAAAGTGC---TTTGCTGCCTTTGACGT-GTTCAATGCAT 471
S. trochoidea SCPC39                GCTTGTGCGTCAAAGTGC---TTTGCTGCCTTTGACGT-GTTCAATGCAT 471
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Scrippsiella._sp._M34*25/5          GCTTGTGTGTCAAAGTGC---TTTGCTGCCTTTGACGC-GTTCAATGCAT 471
S._trochoidea_SZN82                 TCTTGTGCGTCAAAGTGC---TTTGCTGCCTTTGACGT-GTTCAATGCAT 383
S._trochoidea_SZN64                 GCTTGTGCGTCAAAGCGC---TTTGCTGCCTTTGACGT-GTTCAATGCAT 383
C._levantinum_GeoB122               GCTTGTGCGTCAAAGTGC---TTTGATGCCTTTGACGT-GTTTAATGCAT 376
C._levantinum_GeoB*165              GCTTGTGCGTCAAAGTGC---TTTGATGCCTTTGACGT-GTTTAATGCAT 375
S._trochoidea_SZN61                 GCTTGTGCGTCAAAGTGC---TTTGCTGCCTTTGACGC-GTTTAGTGCAT 383
S._trifida_GEoB109                  GCTTGTGTGTCAAAGTGC---CCTGCTGCCTTTGACGC-ATTCAATGCAT 402
Scrippsiella_sp._GeoB*161           GCTTGTGCGTCAAAGTGC---CCTGCTGCCTTTGACGT-GTTCAATGCAT 472
Scrippsiella._sp._D1006             GCTTGTGCGTCAGAGTGC---CTTGCTGCCTCTGATGT-GCTTAACGCAT 452
S._trochoidea_GeoB*214              GCTTGTGCGTCAGAGTGC---CTTGCTGCCTCTGATGT-GCTTAACGCAT 441
S._sp._GeoB138                      GCTTGTGCGTCAGAGTGC---CTTGCTGCCTCTGATGT-GCTTAACGCAT 470
S._trochoidea_GeoB*201              GCTTGTGCGTCAGAGTGC---CTTGTTGCCTCTGATGT-GCTCAACGCAT 441
S._trochoidea_GeoB*200              GCTTGTGCGTCAGAGTGC---CTTGCAGCCTCTGATGT-GCTCAACGCAT 371
S._trochoidea_IO26-01               GCTTGTGCGTCAGAGTGC---CTTGCAGCCTCTGATGT-GCTCAACGCAT 441
Scrippsiella_sp._GeoB188            GCTTGTGCGTCAGAGTGC---CTTGCAGCCTCTGATGT-GCTCAACGCAT 470
Scrippsiella sp.2 SCPC116           GCTTGTGCGTCAGAGTGT---TGTGCTGCCTCTGACGC-GCTCAAAGCAT 464
S._sweeneyae_NIES684                GTTTGTGCGTCAGAGTGC---CCTGCTGCCTCTGATGT-GCTCAATGTGT 383
S. irregularis SCBC17               GTTTGTGCGTTATGGTGC---CTTGCTGCCTTTGACGCGCTTCAATGCAT 467
S. irregularis SCBC19               GTTTGTGCGTTATGGTGC---CTTGCTGCCTTTGACGCGCTTCAATGCAT 467
S._precaria_CS-294                  GCTTGTGCGTCAGAGTGT---CCCGCTGCCTTTGACGTGCTCCAATCCAT 397
S._ramonii_SZN7                     GTTTGTGCGTCAGAGTGC---CCTACTGTCTTTGACGTGCTTCAACTCAA 449
S._hangoei_SHTV1                    GGTTGTGTGTTAAGGTGC---TCTCGGGTCCTTGACGC-ATTCAGAGCAT 408
Peridinium_cinctum_CCAC0102         ACTTGCGTGCCAGGGTGC----CTCCTGCCCCTGGCGC-GTGCAGCAAGC 416
                                       ** *    *  * *           *  **  *

Heterocapsa_pygmaea_CCMP1322        GGGG------ATTCTTTGCTATGCACAACTTGCTAA--GTGTCTTCTGAC 532
Heterocapsa_triquetra_NIES7         GGGG------AGTCTTCACATCGCACAACTTGCAAA--GCATCTT-TGGT 466
Ens.cf._imariensis_JB3              TGGG------ATTTTCCATGATTCACAACTTACCAA--GCATTGC-TGAT 496
Ensi._aff.imariensis_D207           TGGG------ATTTTCCATGATTCACAACTTACCAA--GCATTGC-TGAT 429
Pentapharsodinium_dalei_SZN19       GGGG------ATTTTCCGTGACTTGCAACTTACCAA--GCATTGT-TGAT 495
Ensi._loeblichii_UTEXLB1595         AGGG-------TTTTCCGCGGCTTGCAACTTACCGA--GTGTCCT-TGAC 487
C._albatrosianum_GeoB149            GGGG-----TTTCTTCTGTGTAACACAACTTGCTAA--GCATGTT-ACAT 488
C._albatrosianum_M34-*26/4          GGGG-----TTTCTTCTGTGTAACACAACTTGCTAA--GCATGTT-ACAT 488
Calciodinellum_sp._GeoB120          GGGG-----TTTCTTCTGTGTAACACAACGTGCTAA--GCATGTT-ACAT 488
C._albatrosianum_M34-17             GGGG-----TTTCTTCTGTGTAACACAACTTGCTAA--GCATGTT-ACAT 418
Calciodinellum_operosum_Calope      GGGG-----TTTCTTCTGTGTAACACAACTTGCTAA--GCATGTT-CCAT 508
Scrippsiella_sp._CS-168             GGGG-----ATTTCTCTGTGGCGCTCAACTTGCTAA--GCATCTT-GGAT 521
S._lachrymosa_D192                  GGGG----ATTCCT-CTGTGGCGCACAACTTGCTAA--GCATCTT-TGAT 495
S._lachrymosa_IO25-01               GGGG----ATTCCT-CTGTGGCGCACAACTTGCTAA--GCATCTT-TGAT 416
S._rotunda_SZN66                    AGGGAT---TTCCTCTTGCGGCACGCAACTTGCTAA--ACATCTT-GGAT 515
S._infula_GeoB110                   GGGGAT---TTCCT-TTGCGGCACGCAACTTGCTAA--ACATCTT-GGAT 525
Scrippsiella_sp._GeoB*160           GGGG-----ATTTCTTCATGGCACACACCTTGCTAA--ACATCTC-TGAT 444
S.trochoidea_var.aci_SCCAP499       GGGG-----ACTTCTTCATGGCACACACCTTGCTAA--ACATCTC-TGAT 425
Pernambugia_tuberosa_GeoB61         GGGG-----ATTTCTTTGTGGCACGCAACTTGCTAA--ACATCTT-TGAT 441
S._trochoidea_var.aci_GeoB*213      GGGG-----ATTGCTACTTGGCGCGCAGCTTGCTAA--ACGTTGT-TGAC 444
S.trochoidea_var.aci_GeoB228        GGGG-----ATTGCTACTTGGCGCGCAGCTTGCTAA--ACGTTGT-TGAC 444
S.trochoidea_var.aci_SZN63          GGGG-----ATTGCTACTTGGCGCGCAGCTTGCTAA--ACGTTGT-TGAC 424
S.trochoidea_var.aci_SZN60          GGGG-----ATTGCTACTTGGCGCGCAGCCTGCTAA--ACGTTGT-TGAC 424
Scrippsiella sp. 1 SCPC21           GGAG-----ATGTCACCCTTGTGCTCAACTTGCTAG--ATGTCTC-TGGC 526
S._trochoidea_NIES369               GGGG-----ATTTCTTCTTGGCGCATTACTTGCTAA--ACATCTC-TGAT 514
S._trochoidea_IO14-01               GGGG-----ATTTCTTCTTGGCGCATTACTTGCTAA--ACATCTC-TGAT 415
S._trochoidea_D201                  GGGG-----ATTTCTTCTTGGCGCATTACTTGCGAA--ACATCTC-TGAT 495
S._trochoidea_SZN33                 GGGG-----ATTTCTTCTTGGCGCATTACTTGCGAA--ACATCTC-TGAT 423
S. trochoidea SCBC18                GGGG-----ATTTCCTCTTGGCGCGCAACTTGCTAA--ACGTCGC-TGAC 511
S. trochoidea SCPC36                GGGG-----ATTTCCTCTTGGCGCGCAACTTGCTAA--ACGT-GC-TGAC 466
S. trochoidea SCPC73                GGGG-----ATTTCCTCTTGGCGCGCAACTTGCTAA--ACGTCGC-TGAC 513
S. trochoidea SCPC51                GGGG-----ATTTCCTCTTGGCGCGCAACTTGCTAA--ACGTCGC-TGAC 513
S. trochoidea SCPC39                GGGG-----ATTTCCTCTTGGCGCGCAACTTGCTAA--ACGTCGC-TGAC 513
Scrippsiella._sp._M34*25/5          GGGG-----ATYTCCTCTTGGCGCGCAACTTGCTAA--ACGTCAC-TGAC 513
S._trochoidea_SZN82                 GGGG-----ATTTCCTCTTGGCGCGCAACTTGCTAA--ACGTCGC-TGAC 425
S._trochoidea_SZN64                 GGGG-----ATGTCCTCTTGGCGCACAACTTGCTAA--ACGTCGC-TGAT 425
C._levantinum_GeoB122               GGGG-----ATTTCCTCTTGGCACGCAACTTCCTAA--ACGTCTC-TGAC 418
C._levantinum_GeoB*165              GGGG-----ATTTCCTCTTGGCACGCAACTTCCTAA--ACGTCTC-TGAC 417
S._trochoidea_SZN61                 GGGG-----ATTTCCTCTTGGCGCGCAACCTGCTAA--ACGTCTC-TGAC 425
S._trifida_GEoB109                  GGGG-----ATTTCTTCTTGGTGCGCAACTTGTTAA--ATGTCGC-TGAC 444
Scrippsiella_sp._GeoB*161           TGGG-----GTTTCTTCTTGGCACGCAACTTGCTAA--ACGTCTC-TGAT 514
Scrippsiella._sp._D1006             GGGG-----ATTTCTTCTTGGCGCGCAACTTGTTAA--ACATCTT-CGAT 494
S._trochoidea_GeoB*214              GGGG-----ATTTCTTCTTGGCGCGCAACTTGTTAA--ACATCTT-CGAT 483
S._sp._GeoB138                      GGGG-----ATTTCTTCTTGGCGCGCAACTTGTTAA--ACATCTT-CGAT 512
S._trochoidea_GeoB*201              GGGG-----ATTTCTTCATGGCGCGCAACTTGTTAA--ACATCTT-TGAT 483
S._trochoidea_GeoB*200              GGGG-----ATTTCTTCTTGGCGCGCAACTTGTTAA--ACATCTT-TGAT 413
S._trochoidea_IO26-01               GGGG-----ATTTCTTCTTGGCGCGCAACTTGTTAA--ACATCTT-TGAT 483
Scrippsiella_sp._GeoB188            GGGG-----ATTTCTTCTTGGCGCGCAACTTGTTAA--ACATCTT-TGAT 512
Scrippsiella sp.2 SCPC116           TGGG-----ATCTCTTTGTGGCGAGCAGCTTGCTAA--ACATCTT-TGAT 506
S._sweeneyae_NIES684                GGGG-----ATGTCTTCCAAGCACGCAACTTGCTAA--GCATCTT-GTAT 425
S. irregularis SCBC17               TGGGG----CTCTCTGCGTGGCACACAACTCC-TAA--GCATCCT-TGAT 509
S. irregularis SCBC19               TGGGG----CTCTCTGCGTGGCACACAACTCC-TAA--GCATCCT-TGAT 509
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S._precaria_CS-294                  -GGGG----CTTTTTG--TGGCACGCAACTTTATAA--GCATCTT-TGAT 437
S._ramonii_SZN7                     TGGGA----TTTTTCTTGTGGCACGCAACTTC-TAA--GCATCTT-GGAT 491
S._hangoei_SHTV1                    GGGG-----ATTTCATCTGGTCGCACAACGAATCAT--ACATCTC-TGAT 450
Peridinium_cinctum_CCAC0102         GAGGGCG-AGCAGCATCATGGTAGGCTACAGGCAACC-TGATGCTGTGCC 464
                                       *                   *           *

Heterocapsa_pygmaea_CCMP1322        GCC--TCCTGTTGCTGCATTGGTGAAGGTA--TCCCTTCCATCCAT-AGT 577
Heterocapsa_triquetra_NIES7         GCC--TTCTGTTGCCGTGAATGTGGAGAGA--ACCCTTCCTTCCAT-AGT 511
Ens.cf._imariensis_JB3              GCT--AGTTGTTGTTGGGT-TATTGGAAAGA----CCTGCATGAAG-TAT 538
Ensi._aff.imariensis_D207           GCT--AGTTGTTGTTGGGT-TATTGGAAAGA----CCTGCATGAAG-TAT 471
Pentapharsodinium_dalei_SZN19       GCG--AGTTGTTGCTGGGT-CATTGGAAAGA-GCCTCTGCATGTAG-TAT 540
Ensi._loeblichii_UTEXLB1595         GCT--AGCTGTTGCTGTGCGTGTTGGAAATC-ACTCTCACCTGGAG-TAT 533
C._albatrosianum_GeoB149            GTC--ACTTGTTGTCGT-C-TACCAGGGGAG-CCTCGTGCATGCAG-TAT 532
C._albatrosianum_M34-*26/4          GTC--ACTTGTTGTCGT-C-TACCAGGGGAG-CCTCGTGCATGCAG-YAT 532
Calciodinellum_sp._GeoB120          GTC--ACTTGTTGTCGT-C-TACCAGGGGAG-CCTCGTGCATGCAG-TAT 532
C._albatrosianum_M34-17             GTC--ACTTGTTGTCGT-C-TACCAGGGGAG-CCTCGTGCATGCAG-TAT 462
Calciodinellum_operosum_Calope      GTC--ACTTGTTGTCGT-C-TACCAGGGGAG-CCTCGTGCATGTAG-TAT 552
Scrippsiella_sp._CS-168             GTCATGGTTGTTGG-GTGC-TGTCGGGGGGA-GCTCCTTGATGCAG-TAT 567
S._lachrymosa_D192                  GTA--ATCAGTTGTCGTGC-TCTTGGGGCGA-GCCTCTCGATGTAG-TAT 540
S._lachrymosa_IO25-01               GTA--ATCAGTTGTCGTGC-TCTTGGGGCGA-GCCTCTTGATGTAG-TAT 461
S._rotunda_SZN66                    GTA--ACCTGTTGCTGTGC-TTTCATGGGGG-TCCCTCTGCTGTAG-TAT 560
S._infula_GeoB110                   GTACTACCTGTTGCTGTGC-TTTCTTGGGGG-TCCTCCTGATGTAG-TAT 572
Scrippsiella_sp._GeoB*160           GTA--ACWTGTTGTCGTGC-TTTCGAGGAAA-GCCCCTTGATGCAG-TAT 489
S.trochoidea_var.aci_SCCAP499       GTA--ACTTGTTGTAGTGC-TTTTGGGGAAG-GCCCCTTGATGCAG-TAT 470
Pernambugia_tuberosa_GeoB61         GTC--GTTTGTTGTCGTGC-CTACGGGGAA--GCCCCTCGATGTAG-TAG 485
S._trochoidea_var.aci_GeoB*213      GTA--CCTGGCTGTTGCGC-T-TCGGGGTGA-ACCCCTTGATGTAT-GAT 488
S.trochoidea_var.aci_GeoB228        GTA--CCTGGCTGTTGCGC-T-TCGGGGTGA-ACCCCTTGATGTAT-GAT 488
S.trochoidea_var.aci_SZN63          GTA--CCTGGCTGTTGCGC-T-TCGGGGTGA-ACCCCTTGATGTAT-GAT 468
S.trochoidea_var.aci_SZN60          GTA--CCTGGCTGTTGCGC-T-TCGGGGTGA-ACCCCTTGATGTAT-GAT 468
Scrippsiella sp. 1 SCPC21           ACA--ACTCGTTGGTGCAC-T-TGGGTGTAA-ACCCCTTGATGTAT-TGT 570
S._trochoidea_NIES369               GTA--ACTTGTTGTTGCGC-T-TTGGGGCAA-TTCCTTTGATGTAT-TGK 558
S._trochoidea_IO14-01               GTA--ACTTGTTGTTGCGC-T-TTGGGGCAA-TTCCTTTGATGTAT-TGT 459
S._trochoidea_D201                  GTA--ACTTGTTGTTGTGC-T-TTGGGGCAA-TTCCTTTGATGTAT-TGT 539
S._trochoidea_SZN33                 GTA--ACTTGTTGTTGTGC-T-TTGGGGCAA-TTCCTTTGATGTAT-TGT 467
S. trochoidea SCBC18                GCA--ACTTGTTGTTGTGC-T-TTGGGGCAA-CCTCCTTGATTCA--TAT 554
S. trochoidea SCPC36                GCA--ACTTGTTGTTGTGC-T-TTGGGGCAA-CCTCCTTGATTCA--TAT 508
S. trochoidea SCPC73                GCC--ACCTGTTGTTGTGC-T-TTGGGGCAA-CCTCCTTGATTCA--TAT 556
S. trochoidea SCPC51                GCA--ACTTGTTGTTGTGC-T-TGGGGGCAA-CCTCCCTGATTCA--TAT 556
S. trochoidea SCPC39                GCA--ACTTGTTGTTGTGC-T-TTGGGGCAA-CCTCCTTGATTCA--TAT 556
Scrippsiella._sp._M34*25/5          GCA--ACTTGTTGTTGTGC-T-TTGGGGCAA-CCTCCTTGATTCA--TAT 556
S._trochoidea_SZN82                 GCA--ACTTGTTGTTGTGC-T-TTGGGGCAA-CCTCCTTGATTCA--TAT 468
S._trochoidea_SZN64                 GTA--ACTTGTTGTTGTGC-T-TTGGGGCAA-CCTCCTTGATTCA--TAT 468
C._levantinum_GeoB122               GTA--ACTTGTTGTTGTGC-C-CTGGGGCAA-CCTCCTTGATTTG--TAT 461
C._levantinum_GeoB*165              GTA--ACATGCTGGTGTGC-C-CTGGGGCAA-CCTCCTTGATGTG--TAT 460
S._trochoidea_SZN61                 GTA--ACTTGTTGTTGCGC-C-TTGGGGCAA-CCTCCTTGATTCA--TAT 468
S._trifida_GEoB109                  GTC--ACTTGTTGTTGCGC-C-TTGGGGCAA--ACCCTTTATGTAT-TAT 487
Scrippsiella_sp._GeoB*161           GTA--ACTTGTTGTTGTGC-T-TTGGGGCAA-CCTCCTTGATGTAG-TAT 558
Scrippsiella._sp._D1006             GTA--ACCTGCTGCTGCGC-TTTTGGGGAGA-ACCCCTTGATGTAG-TAT 539
S._trochoidea_GeoB*214              GTA--ACCTGCTGCTGCGC-TTTTGGGGAGA-ACCCCTTGATGTAG-TAT 528
S._sp._GeoB138                      GTA--ACCTGCTGCTGCGC-TTTTGGGGAGA-ACCCCTTGATGTAG-TAT 557
S._trochoidea_GeoB*201              GTA--GCCTGCTGCTGCGC-TTTTGGGGAGA-ACCCCTTGATGTAG-TAT 528
S._trochoidea_GeoB*200              GTA--ACCTGCTGCTGTGC-TTTTGGGGAGA-ACCCCTTGATGTAG-TAT 458
S._trochoidea_IO26-01               GTA--ACCTGCTGCTGTGC-TTTTGGGGAGA-ACCCCTTGATGTAG-TAT 528
Scrippsiella_sp._GeoB188            GTA--ACCTGCTGCTGTGC-TTTTGGGGAGA-ACCCCTTGATGTAG-TAT 557
Scrippsiella sp.2 SCPC116           GTA--ACTTGCTGTTGTGC-TGCTGGGGAGA-GCCCCTCGATGTAG-GGT 551
S._sweeneyae_NIES684                GTA--ACTTGTTGTTGTGC-T-CTGGGGAGA-GCCCTTTGATGTAG-TAT 469
S. irregularis SCBC17               GTC--TTGTGTTGGTGTGC-TCTCAAGGAGAGAACCATTGATGTAG-TAT 555
S. irregularis SCBC19               GTC--TTGTGTTGGTGTGC-TCTCAAGGAGAGAACCATTGATGCAG-TAT 555
S._precaria_CS-294                  GTC--TTGTGTTGATGTGC-TTTCMAGGAGAGAACCCTTGATGCAG-TAT 483
S._ramonii_SZN7                     GTC--TTATGTTGACGTGC-TTTCAAGGAGAGAACCCTTGATGCAG-TAT 537
S._hangoei_SHTV1                    GTT--GCTTGTTGGTGCAT-GTCCGGAGGGA--ACCCTTAGTTCGAGTAT 495
Peridinium_cinctum_CCAC0102         TGCGAGGGCGTGGCTGCGA-T---GGGGAGCCCTCCCTGTATCCGA-CAT 509
                                          *  *  *                    *

Heterocapsa_pygmaea_CCMP1322        CCTTCTGGGACTTC---------------------------------- 591
Heterocapsa_triquetra_NIES7         CACT-TGTGACTTC---------------------------------- 524
Ens.cf._imariensis_JB3              TTA---AGCACTTGTT-------------------------------- 551
Ensi._aff.imariensis_D207           TTA---AGCACTTGTTA------------------------------- 485
Pentapharsodinium_dalei_SZN19       TTC--TAATACTTCTC-------------------------------- 554
Ensi._loeblichii_UTEXLB1595         GTC--TAATACTTCCC-------------------------------- 547
C._albatrosianum_GeoB149            CTC--GCATACTCCTK-------------------------------- 546
C._albatrosianum_M34-*26/4          CTC--GCATACTCCTG-------------------------------- 546
Calciodinellum_sp._GeoB120          CTC--GCATACTCCSTG------------------------------- 547
C._albatrosianum_M34-17             CTC--GCATACTCCTTG------------------------------- 477
Calciodinellum_operosum_Calope      CTC--GCATACTCCTT-------------------------------- 566
Scrippsiella_sp._CS-168             GGT-CTCATACTTCTGAAGACATGAAG--------------------- 593
S._lachrymosa_D192                  GGC-TTG-TACTTTTG-------------------------------- 554
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S._lachrymosa_IO25-01                GGC-TTGGTACTTTTG---------------------------------- 476
S._rotunda_SZN66                     CAC--GCATACTC------------------------------------- 571
S._infula_GeoB110                    CACTCGCATACTCCATGAAGACATGAAG---------------------- 600
Scrippsiella_sp._GeoB*160            GTT--TCATACTTCCGAAGACATGAAG---------------------- 514
S.trochoidea_var.aci_SCCAP499        GTC--TCGTACTT------------------------------------- 481
Pernambugia_tuberosa_GeoB61          GCT--TCCTACTTCTGAAGACATGAAG---------------------- 510
S._trochoidea_var.aci_GeoB*213       GTC--TTATACATGTGAAGACATGAAG---------------------- 513
S.trochoidea_var.aci_GeoB228         GTC--TTATACATGTGAAGACATGAAG---------------------- 513
S.trochoidea_var.aci_SZN63           GTC--TTATACAT------------------------------------- 479
S.trochoidea_var.aci_SZN60           GTC--TTATACAT------------------------------------- 479
Scrippsiella sp. 1 SCPC21            GTC--TTCCACCTGCAAACTAAGTCAGG---------------------- 596
S._trochoidea_NIES369                ATY--TCATACATTGAAGACATGAAG---------------------- 582
S._trochoidea_IO14-01                ATT--TCATACATTTG---------------------------------- 473
S._trochoidea_D201                   ATT--TCATACATGCG---------------------------------- 553
S._trochoidea_SZN33                  ATT--TCATACAT------------------------------------- 478
S. trochoidea SCBC18                 GTT--TCATACATGTGAAGACATGAAGT---------------------- 580
S. trochoidea SCPC36                 GTT--TCAT----------------------------------------- 515
S. trochoidea SCPC73                 GTT--TCATA---------------------------------------- 564
S. trochoidea SCPC51                 GTT--TCATACATGTGAAGACATGAAGT---------------------- 582
S. trochoidea SCPC39                 GTT--TCATACATGTGAAGACATGAAGT---------------------- 582
Scrippsiella._sp._M34*25/5           GTT--TCATACATGTGAAGACATGAAG---------------------- 581
S._trochoidea_SZN82                  GTT--TCATACAT------------------------------------- 479
S._trochoidea_SZN64                  GTT--TCATACAT------------------------------------- 479
C._levantinum_GeoB122                GTT--GCATACATGTG---------------------------------- 475
C._levantinum_GeoB*165               GTT--GCATACATGT----------------------------------- 473
S._trochoidea_SZN61                  GTT--TCATACAT------------------------------------- 479
S._trifida_GEoB109                   GTT--TCATAAATGTGAAGACATGAAG---------------------- 512
Scrippsiella_sp._GeoB*161            GCT--TCATACATGCGAAGACATGAAG---------------------- 583
Scrippsiella._sp._D1006              GTC--TCATACATCCG---------------------------------- 553
S._trochoidea_GeoB*214               GTC--TCATACATCCG---------------------------------- 542
S._sp._GeoB138                       GTC--TCATACATCCGAAGACATGAAG---------------------- 582
S._trochoidea_GeoB*201               GTC--TCATACATCCG---------------------------------- 542
S._trochoidea_GeoB*200               GTT--TCATACATCCG---------------------------------- 472
S._trochoidea_IO26-01                GTT--TCATACATCCG---------------------------------- 542
Scrippsiella_sp._GeoB188             GTT--TCATACATCCGAAGACATGAAG---------------------- 582
Scrippsiella sp.2 SCPC116            GTT--TCACCCTTCTTAAGA----------------------------- 569
S._sweeneyae_NIES684                 GAC--TCATACATCTG---------------------------------- 483
S. irregularis SCBC17                GCT--GCTTACCTTTGAAGACATGAAGTCAAGTCAGCAAACCCGCTGACT 603
S. irregularis SCBC19                GCT--GCTTACCTTTGAAGACATGAAGTCAAGTCAGCAAACCCGCTGACT 603
S._precaria_CS-294                   GGY--TCATACCTCTGAAGACATGAAG---------------------- 508
S._ramonii_SZN7                      GGC--TCATACCT------------------------------------- 548
S._hangoei_SHTV1                     TGC--TTATACCTTTCAAGACATGAAG---------------------- 520
Peridinium_cinctum_CCAC0102          GAAG---------------------------------------------- 513

Heterocapsa_pygmaea_CCMP1322         ----------------------------------------------------
Heterocapsa_triquetra_NIES7          ----------------------------------------------------
Ens.cf._imariensis_JB3               ----------------------------------------------------
Ensi._aff.imariensis_D207            ----------------------------------------------------
Pentapharsodinium_dalei_SZN19        ----------------------------------------------------
Ensi._loeblichii_UTEXLB1595          ----------------------------------------------------
C._albatrosianum_GeoB149             ----------------------------------------------------
C._albatrosianum_M34-*26/4           ----------------------------------------------------
Calciodinellum_sp._GeoB120           ----------------------------------------------------
C._albatrosianum_M34-17              ----------------------------------------------------
Calciodinellum_operosum_Calope       ----------------------------------------------------
Scrippsiella_sp._CS-168              ----------------------------------------------------
S._lachrymosa_D192                   ----------------------------------------------------
S._lachrymosa_IO25-01                ----------------------------------------------------
S._rotunda_SZN66                     ----------------------------------------------------
S._infula_GeoB110                    ----------------------------------------------------
Scrippsiella_sp._GeoB*160            ----------------------------------------------------
S.trochoidea_var.aci_SCCAP499        ----------------------------------------------------
Pernambugia_tuberosa_GeoB61          ----------------------------------------------------
S._trochoidea_var.aci_GeoB*213       ----------------------------------------------------
S.trochoidea_var.aci_GeoB228         ----------------------------------------------------
S.trochoidea_var.aci_SZN63           ----------------------------------------------------
S.trochoidea_var.aci_SZN60           ----------------------------------------------------
Scrippsiella sp. 1 SCPC21            ----------------------------------------------------
S._trochoidea_NIES369                ----------------------------------------------------
S._trochoidea_IO14-01                ----------------------------------------------------
S._trochoidea_D201                   ----------------------------------------------------
S._trochoidea_SZN33                  ----------------------------------------------------
S. trochoidea SCBC18                 ----------------------------------------------------
S. trochoidea SCPC36                 ----------------------------------------------------
S. trochoidea SCPC73                 ----------------------------------------------------
S. trochoidea SCPC51                 ----------------------------------------------------
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Scrippsiella_sp._SCPC39          ----------------------------------------------------
Scrippsiella._sp._M34*25/5       ----------------------------------------------------
S._trochoidea_SZN82              ----------------------------------------------------
S._trochoidea_SZN64              ----------------------------------------------------
C._levantinum_GeoB122            ----------------------------------------------------
C._levantinum_GeoB*165           ----------------------------------------------------
S._trochoidea_SZN61              ----------------------------------------------------
S._trifida_GEoB109               ----------------------------------------------------
Scrippsiella_sp._GeoB*161        ----------------------------------------------------
Scrippsiella._sp._D1006          ----------------------------------------------------
S._trochoidea_GeoB*214           ----------------------------------------------------
S._sp._GeoB138                   ----------------------------------------------------
S._trochoidea_GeoB*201           ----------------------------------------------------
S._trochoidea_GeoB*200           ----------------------------------------------------
S._trochoidea_IO26-01            ----------------------------------------------------
Scrippsiella_sp._GeoB188         ----------------------------------------------------
Scrippsiella sp.2 SCPC116        ----------------------------------------------------
S._sweeneyae_NIES684             ----------------------------------------------------
S. irregularis SCBC17            TAAGCATCTGTCAG---------------------------------- 617
S. irregularis SCBC19            TAAGCATCTGTCAG---------------------------------- 616
S._precaria_CS-294               ----------------------------------------------------
S._ramonii_SZN7                  ----------------------------------------------------
S._hangoei_SHTV1                 ----------------------------------------------------
Peridinium_cinctum_CCAC0102      ----------------------------------------------------

Heterocapsa_pygmaea_CCMP1322     -------
Heterocapsa_triquetra_NIES7      -------
Ens.cf._imariensis_JB3           -------
Ensi._aff.imariensis_D207        -------
Pentapharsodinium_dalei_SZN19    -------
Ensi._loeblichii_UTEXLB1595      -------
C._albatrosianum_GeoB149         -------
C._albatrosianum_M34-*26/4       -------
Calciodinellum_sp._GeoB120       -------
C._albatrosianum_M34-17          -------
Calciodinellum_operosum_Calope   -------
Scrippsiella_sp._CS-168          -------
S._lachrymosa_D192               -------
S._lachrymosa_IO25-01            -------
S._rotunda_SZN66                 -------
S._infula_GeoB110                -------
Scrippsiella_sp._GeoB*160        -------
S.trochoidea_var.aci_SCCAP499    -------
Pernambugia_tuberosa_GeoB61      -------
S._trochoidea_var.aci_GeoB*213   -------
S.trochoidea_var.aci_GeoB228     -------
S.trochoidea_var.aci_SZN63       -------
S.trochoidea_var.aci_SZN60       -------
Scrippsiella sp. 1 SCPC21        -------
S._trochoidea_NIES369            -------
S._trochoidea_IO14-01            -------
S._trochoidea_D201               -------
S._trochoidea_SZN33              -------
Scrippsiella_sp_SCBC18           -------
Scrippsiella_sp._SCPC36          -------
scrippsiella_sp._SCPC73          -------
Scrippsiella._sp.SCPC51          -------
Scrippsiella_sp._SCPC39          -------
Scrippsiella._sp._M34*25/5       -------
S._trochoidea_SZN82              -------
S._trochoidea_SZN64              -------
C._levantinum_GeoB122            -------
C._levantinum_GeoB*165           -------
S._trochoidea_SZN61              -------
S._trifida_GEoB109               -------
Scrippsiella_sp._GeoB*161        -------
Scrippsiella._sp._D1006          -------
S._trochoidea_GeoB*214           -------
S._sp._GeoB138                   -------
S._trochoidea_GeoB*201           -------
S._trochoidea_GeoB*200           -------
S._trochoidea_IO26-01            -------
Scrippsiella_sp._GeoB188         -------
Sscrippsiella_SCPC116            -------
S._sweeneyae_NIES684             -------
S. irregularis SCBC17            -------
```

```
S. irregularis SCBC19              -------
S._precaria_CS-294                 -------
S._ramonii_SZN7                    -------
S._hangoei_SHTV1                   -------
Peridinium_cinctum_CCAC0102        -------
```

Figure3 . Sequence alignment of partial rDNA -LSU of raphidophyte species

```
Chattonella_subsalsaAF210736        TTGCCGTT-GCCTGTGCGTTCCTCTCCCGTTGCTGTCTCTGTTCTACTGC 49
Chattonella_subsalsaAF409126        TTGCCGTT-GCCTGTGCGTTCCTCTCCCGTTGCTGTCTCTGTTCTACTGC 49
Chattonella cf. subsalsa CHPI36     TTGCCGTT-GCCTATGCGTTACTCTCCGATTGCTGTCTCGGTTCTACTGC 49
Chattonella_antiquaAF210737         TTGCCGTT-GCCTGCTTGTTACTCTCCTGTTGCTGTTTCTGTCCTACTGC 49
Chattonella_marinaAF210739          TTGCCGTT-GCCTGCTTGTTACTCTCCTGTTGCTGTTTCTGTCCTACTGC 49
Chattonella_ovataAF210738           TTGCCGTT-GCCTGCTTGTTACTCTCCTGTTGCTGTTTCTGTCCTACTGC 49
Chattonella_marinaAY704162          TTGCCGTT-GCCTGCTTGTTACTCTCCTGTTGCTGTTTCTGTCCTACTGC 49
Chattonella_ovataAY704163           TTGCCGTT-GCCTGCTTGTTACTCTCCTGTTGCTGTTTCTGTCCTACTGC 49
Vacuolaria_virescensAF210742        TCGCCGTTTGCCTGCCCGTCGCTCTCTGCCGGTTGTTGCTGTCCTACTGC 50
Vacuolaria_virescensAF409125        TCGCCGTTTGCCTGCCCGTCGCTCTCTGCCGGTTGTTGCTGTCCTACTGC 50
Heterosigma_akashiwo_AF210741       TGCTCCTTTGACTGCGCGTTCCTCTCTCGGGTATGCTGGTGTC-TACTGC 49
Heterosigma_akashiwoAF086948        TGCTCCTTTGACTGCGCGTTCCTCTCTCGGGTATGCTGGTGTC-TACTGC 49
Heterosigma_akashiwoAY704161        TGCTCCTTTGACTGCGCGTTCCTCTCTCGGGTATGCTGGTGTC-TACTGC 49
Heterosigma_akashiwoAF042820        TGCTCCTTTGACTGCGCGTTCCTCTCTCGGGTATGCTGGTGTC-TACTGC 49
Olisthodiscus_luteusAF210743        AGGGCGCGCGCAAGCGACATCCTGACAAATGATTCTTCCACCGA------ 44
Cylindrotheca_closteriumAF4176      TCTTTACCCCGTCTTGAAACACGGACCAAGGAGTCTAACATATGTGCGAG 50
                                                    *   *       *

Chattonella_subsalsaAF210736        T-TGCAGTGCTCAGTTGCAGTAGTTGGACTGTGCGTATTATGCATGCAAG 98
Chattonella_subsalsaAF409126        T-TGCAGTGCTCAGTTGCAGTAGTTGGACTGTGCGTATTATGCATGCAAG 98
Chattonella cf. subsalsa CCHPI36    T-TGCAGTGCTCAGTGGCAGTAGTTGGACTGTGCGTATTACGCATGCAAG 98
Chattonella_antiquaAF210737         T-TGCAGTGTTCGGTTGCAGTGATTGGACTGTGCAAGTTATGCATGCAAG 98
Chattonella_marinaAF210739          T-TGCAGTGTTCGGTTGCAGTGATTGGACTGTGCAAGTTATGCATGCAAG 98
Chattonella_ovataAF210738           T-TGCAGTGTTCGGTTGCAGTGATTGGACTGTGCAAGTTATGCATGCAAG 98
Chattonella_marinaAY704162          T-TGCAGTGTTCGGTTGCAGTGATTGGACTGTGCAAGTTATGCATGCAAG 98
Chattonella_ovataAY704163           T-TGCAGTGTTCGGTTGCAGTGATTGGACTGTGCAAGTTATGCATGCAAG 98
Vacuolaria_virescensAF210742        T-TGCAGTGCTCAGCTGCAGCTGACTGACTGTGCGGGTCATGCATGCGAG 99
Vacuolaria_virescensAF409125        T-TGCAGTGCTCAGCTGCAGCTGACTGACTGTGCGGGTCATGCATGCGAG 99
Heterosigma_akashiwo_AF210741       T-TGCAGTTTTCATTTTCATGCTTGCGACTGTGCGTGTTATTCATGAGCG 98
Heterosigma_akashiwoAF086948        T-TGCAGTTTTCATTTTCATGCTTGCCACTGTGCGTGTTATTCATGAGCG 98
Heterosigma_akashiwoAY704161        T-TGCAGTTTTCATTTTCATGCTTGCGACTGTGCGTGTTATTCATGAGCG 98
Heterosigma_akashiwoAF042820        T-TGCAGTTTTCATTTTCATGCTTGCGACTGTGCGTGTTATTCATGAGCG 98
Olisthodiscus_luteusAF210743        ---------------------------------------------------
Cylindrotheca_closteriumAF4176      TACAGGGGTGTCAAACCCCCGTGCGGAATGAAAGTGACAGTGGTTGGACC 100

Chattonella_subsalsaAF210736        GTCAGGATCCTGACGAA-TGGCTTTATTCACCCGCAA------------- 134
Chattonella_subsalsaAF409126        GTCAGGATCCTGACGAAATGGCTTTATTTACCCCGTCTTGAAACACGGAC 148
Chattonella cf. subsalsa CHPI36     GTCAGGATCCTGACGAAATGGCTTTATTTACCCCGTCTTGAAACACGGAC 148
Chattonella_antiquaAF210737         GTCAGGATCCTGACGAA-TGGCTTTATTAACCCGAA-------------- 133
Chattonella_marinaAF210739          GTCAGGATCCTGACGAA-TGGCTTTATTAACCCGAA-------------- 133
Chattonella_ovataAF210738           GTCAGGATCCTGACGAA-TGGCTTTATTAACCCGAA-------------- 133
Chattonella_marinaAY704162          GTCAGGATCCTGACGAA-AGGCTTTATTT--------------------- 126
Chattonella_ovataAY704163           GTCAGGATCCTGACGAA-AGGCTTTATTT--------------------- 126
Vacuolaria_virescensAF210742        GTCAGGATCCTGA-GGACTGGCCGTAATAACCCAA--------------- 133
Vacuolaria_virescensAF409125        GTCAGGATCCTGACGAAATGGCTTTATTTACCCCGTCTTGAAACACGGAC 149
Heterosigma_akashiwo_AF210741       AACATGATGTTGAAGAAATGGCTTTAATTACCCCGTCTTGAAACACGGAC 148
Heterosigma_akashiwoAF086948        AACATGATGTTGAAGAAATGGCTTTAATTACCCCGTCTTGAAACACGGAC 148
Heterosigma_akashiwoAY704161        AACATGATGTTGAAGAAATGGCTTTAATTA--------------------- 128
Heterosigma_akashiwoAF042820        AACATGATGTTGAAGAAATGGCTTTAATTACCCCGTCTTGAAACACGGAC 148
Olisthodiscus_luteusAF210743        ---------------------------------------------------
Cylindrotheca_closteriumAF4176      TTTTTGGGCACAATCCGCCGGCCTCAATCCTTCGGGAGAACGGTCTGAGT 150

Chattonella_subsalsaAF210736        ---------------------------------------------------
Chattonella_subsalsaAF409126        CAAGGAGTCTAACATACGTGCGAGTACTTTGGTGACAAACCAATGTGCGC 198
Chattonella cf. subsalsa CHPI36     CAAGGAGTCTAACATACGTGCGAGTACTTTGGTGGCAAACCAATGTGCGC 198
Chattonella_antiquaAF210737         ---------------------------------------------------
Chattonella_marinaAF210739          ---------------------------------------------------
Chattonella_ovataAF210738           ---------------------------------------------------
Chattonella_marinaAY704162          ---------------------------------------------------
Chattonella_ovataAY704163           ---------------------------------------------------
Vacuolaria_virescensAF210742        ---------------------------------------------------
Vacuolaria_virescensAF409125        CAAGGAGTCTAACATACGTGCGAGTACTTTGGTG---------------- 183
Heterosigma_akashiwo_AF210741       CAAGG-------------------------------------------- 153
Heterosigma_akashiwoAF086948        CAAG--------------------------------------------- 152
Heterosigma_akashiwoAY704161        ---------------------------------------------------
Heterosigma_akashiwoAF042820        CAAGG-------------------------------------------- 153
Olisthodiscus_luteusAF210743        ---------------------------------------------------
Cylindrotheca_closteriumAF4176      GTGAGCATACATGTTGGGACCCGAAAGATGGTGAACTATGCCTGAATAGG 200

Chattonella_subsalsaAF210736        ---------------------------------------------------
Chattonella_subsalsaAF409126        AATGAAAGTAAAGGCTGGCTTCCGTCAGCTCAGGTGGGATCCGTTTGGAG 248
```

```
Chattonella cf. subsalsa CHPI36     AATGAAAGTAAAGGCTGGCTTCCGTCAGCTCATGTGGGATCCTTATGGAG 248
Chattonella_antiquaAF210737         --------------------------------------------------
Chattonella_marinaAF210739          --------------------------------------------------
Chattonella_ovataAF210738           --------------------------------------------------
Chattonella_marinaAY704162          --------------------------------------------------
Chattonella_ovataAY704163           --------------------------------------------------
Vacuolaria_virescensAF210742        --------------------------------------------------
Vacuolaria_virescensAF409125        --------------------------------------------------
Heterosigma_akashiwo_AF210741       --------------------------------------------------
Heterosigma_akashiwoAF086948        --------------------------------------------------
Heterosigma_akashiwoAY704161        --------------------------------------------------
Heterosigma_akashiwoAF042820        --------------------------------------------------
Olisthodiscus_luteusAF210743        --------------------------------------------------
Cylindrotheca_closteriumAF4176      GTGAAGCCAGGGGAAACTCTGGTGGAGGCTCGTAGCGATTCTGACGTGCA 250

Chattonella_subsalsaAF210736        --------------------------------------------------
Chattonella_subsalsaAF409126        CACCATCGACCGACCATTATGCCTTCGGGCTAAAGGTTTGAGTGTGAGCA 298
Chattonella cf. subsalsa CHPI36     CACCATCGACCGACCATTATGCCTTCGGGCTAAAGGTTTGAGTGTGAGCA 298
Chattonella_antiquaAF210737         --------------------------------------------------
Chattonella_marinaAF210739          --------------------------------------------------
Chattonella_ovataAF210738           --------------------------------------------------
Chattonella_marinaAY704162          --------------------------------------------------
Chattonella_ovataAY704163           --------------------------------------------------
Vacuolaria_virescensAF210742        --------------------------------------------------
Vacuolaria_virescensAF409125        --------------------------------------------------
Heterosigma_akashiwo_AF210741       --------------------------------------------------
Heterosigma_akashiwoAF086948        --------------------------------------------------
Heterosigma_akashiwoAY704161        --------------------------------------------------
Heterosigma_akashiwoAF042820        --------------------------------------------------
Olisthodiscus_luteusAF210743        --------------------------------------------------
Cylindrotheca_closteriumAF4176      AATCGATCGTCAAATTTGGG---------------------------- 270

Chattonella_subsalsaAF210736        --------------------------------------------------
Chattonella_subsalsaAF409126        TTTATGTTGGGACCCGAAAGATGGTGAACTATGCCTGAATAGGGCGAAGC 348
Chattonella cf. subsalsa CHPI36     TTTATGTTGGGACCCGAAAGATGGTGAACTATGCCTGAATAGGGCGAAGC 348
Chattonella_antiquaAF210737         --------------------------------------------------
Chattonella_marinaAF210739          --------------------------------------------------
Chattonella_ovataAF210738           --------------------------------------------------
Chattonella_marinaAY704162          --------------------------------------------------
Chattonella_ovataAY704163           --------------------------------------------------
Vacuolaria_virescensAF210742        --------------------------------------------------
Vacuolaria_virescensAF409125        --------------------------------------------------
Heterosigma_akashiwo_AF210741       --------------------------------------------------
Heterosigma_akashiwoAF086948        --------------------------------------------------
Heterosigma_akashiwoAY704161        --------------------------------------------------
Heterosigma_akashiwoAF042820        --------------------------------------------------
Olisthodiscus_luteusAF210743        --------------------------------------------------
Cylindrotheca_closteriumAF4176      --------------------------------------------------

Chattonella_subsalsaAF210736        --------------------------------------------------
Chattonella_subsalsaAF409126        CAGGGGAAACTCTGGTGGAGGCTCGTAGCGATTCTGACGTGCAAATCGAT 398
Chattonella cf. subsalsa CHPI36     CAGGGGAAACTCTGGTGGAGGCTCGTAGCGATTCTGACGTGCAAATCGAT 398
Chattonella_antiquaAF210737         --------------------------------------------------
Chattonella_marinaAF210739          --------------------------------------------------
Chattonella_ovataAF210738           --------------------------------------------------
Chattonella_marinaAY704162          --------------------------------------------------
Chattonella_ovataAY704163           --------------------------------------------------
Vacuolaria_virescensAF210742        --------------------------------------------------
Vacuolaria_virescensAF409125        --------------------------------------------------
Heterosigma_akashiwo_AF210741       --------------------------------------------------
Heterosigma_akashiwoAF086948        --------------------------------------------------
Heterosigma_akashiwoAY704161        --------------------------------------------------
Heterosigma_akashiwoAF042820        --------------------------------------------------
Olisthodiscus_luteusAF210743        --------------------------------------------------
Cylindrotheca_closteriumAF4176      --------------------------------------------------

Chattonella_subsalsaAF210736        --------------------------------------------------
Chattonella_subsalsaAF409126        CGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCT 448
Chattonella cf. subsalsa CHPI36     CGTCAAATTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCT 448
Chattonella_antiquaAF210737         --------------------------------------------------
Chattonella_marinaAF210739          --------------------------------------------------
Chattonella_ovataAF210738           --------------------------------------------------
Chattonella_marinaAY704162          --------------------------------------------------
```

```
Chattonella_ovataAY704163          ------------------------------------------------
Vacuolaria_virescensAF210742       ------------------------------------------------
Vacuolaria_virescensAF409125       ------------------------------------------------
Heterosigma_akashiwo_AF210741      ------------------------------------------------
Heterosigma_akashiwoAF086948       ------------------------------------------------
Heterosigma_akashiwoAY704161       ------------------------------------------------
Heterosigma_akashiwoAF042820       ------------------------------------------------
Olisthodiscus_luteusAF210743       ------------------------------------------------
Cylindrotheca_closteriumAF4176     ------------------------------------------------

Chattonella_subsalsaAF210736       ------------------------------------------------
Chattonella_subsalsaAF409126       GGTTCCCTCCGAAATTTCCCTCAGGATAGCTGCAACCGGTTAAGAGTTTT 498
Chattonella cf. subsalsa CHPI36    GGTTCCCTCCGAAATTTCCCTCAGGATAGCTGCAACCGGTTAAGAGTTTT 498
Chattonella_antiquaAF210737        ------------------------------------------------
Chattonella_marinaAF210739         ------------------------------------------------
Chattonella_ovataAF210738          ------------------------------------------------
Chattonella_marinaAY704162         ------------------------------------------------
Chattonella_ovataAY704163          ------------------------------------------------
Vacuolaria_virescensAF210742       ------------------------------------------------
Vacuolaria_virescensAF409125       ------------------------------------------------
Heterosigma_akashiwo_AF210741      ------------------------------------------------
Heterosigma_akashiwoAF086948       ------------------------------------------------
Heterosigma_akashiwoAY704161       ------------------------------------------------
Heterosigma_akashiwoAF042820       ------------------------------------------------
Olisthodiscus_luteusAF210743       ------------------------------------------------
Cylindrotheca_closteriumAF4176     ------------------------------------------------

Chattonella_subsalsaAF210736       ------------------------------------------------
Chattonella_subsalsaAF409126       ATCAGGTAAAGCGAATGATTAGAGGCCTCGGGGTCGGAATGACCTCGACC 548
Chattonella cf. subsalsa CHPI36    ATCAGGTAAAGCGAATGATTAGAGGCCTCGGGGTCGGAATGACCTCGACC 548
Chattonella_antiquaAF210737        ------------------------------------------------
Chattonella_marinaAF210739         ------------------------------------------------
Chattonella_ovataAF210738          ------------------------------------------------
Chattonella_marinaAY704162         ------------------------------------------------
Chattonella_ovataAY704163          ------------------------------------------------
Vacuolaria_virescensAF210742       ------------------------------------------------
Vacuolaria_virescensAF409125       ------------------------------------------------
Heterosigma_akashiwo_AF210741      ------------------------------------------------
Heterosigma_akashiwoAF086948       ------------------------------------------------
Heterosigma_akashiwoAY704161       ------------------------------------------------
Heterosigma_akashiwoAF042820       ------------------------------------------------
Olisthodiscus_luteusAF210743       ------------------------------------------------
Cylindrotheca_closteriumAF4176     ------------------------------------------------

Chattonella_subsalsaAF210736       ------------------------------------------------
Chattonella_subsalsaAF409126       TATTCTCAAACTTTAAATGGGTAAGAACCCGGGGTTGCTTAATTGAACCC 598
Chattonella cf. subsalsa CHPI36    TATTCTCAAACTTTAAATGGGTAAGAACCCGGGGTTGCTTAATTGAACCC 598
Chattonella_antiquaAF210737        ------------------------------------------------
Chattonella_marinaAF210739         ------------------------------------------------
Chattonella_ovataAF210738          ------------------------------------------------
Chattonella_marinaAY704162         ------------------------------------------------
Chattonella_ovataAY704163          ------------------------------------------------
Vacuolaria_virescensAF210742       ------------------------------------------------
Vacuolaria_virescensAF409125       ------------------------------------------------
Heterosigma_akashiwo_AF210741      ------------------------------------------------
Heterosigma_akashiwoAF086948       ------------------------------------------------
Heterosigma_akashiwoAY704161       ------------------------------------------------
Heterosigma_akashiwoAF042820       ------------------------------------------------
Olisthodiscus_luteusAF210743       ------------------------------------------------
Cylindrotheca_closteriumAF4176     ------------------------------------------------

Chattonella_subsalsaAF210736       ------------------------------------------------
Chattonella_subsalsaAF409126       TGCGGTCGAATCTCGGTTGCTAGTGGGCCAGTCCGGGTAAGCTGGACTGG 648
Chattonella cf. subsalsa CHPI36    TGCGGTCGAATCTCGGTTGCTAGTGGGCCAGTCCGGGTAAGCTGGACTGG 648
Chattonella_antiquaAF210737        ------------------------------------------------
Chattonella_marinaAF210739         ------------------------------------------------
Chattonella_ovataAF210738          ------------------------------------------------
Chattonella_marinaAY704162         ------------------------------------------------
Chattonella_ovataAY704163          ------------------------------------------------
Vacuolaria_virescensAF210742       ------------------------------------------------
Vacuolaria_virescensAF409125       ------------------------------------------------
Heterosigma_akashiwo_AF210741      ------------------------------------------------
Heterosigma_akashiwoAF086948       ------------------------------------------------
```

```
Heterosigma_akashiwoAY704161        ----------------------------------------------------
Heterosigma_akashiwoAF042820        ----------------------------------------------------
Olisthodiscus_luteusAF210743        ----------------------------------------------------
Cylindrotheca_closteriumAF4176      ----------------------------------------------------

Chattonella_subsalsaAF210736        ----------------------------------------------------
Chattonella_subsalsaAF409126        CGATGCGGGATGATCCGAACGCTTAGTTAAAGTGCCTAACTGCTCGCTCA 698
Chattonella cf. subsalsa CHPI36     CGATGCGGGATGATCCGAACGCTTAGTTAAAGTGCCTAACTGCTCGCTCA 698
Chattonella_antiquaAF210737         ----------------------------------------------------
Chattonella_marinaAF210739          ----------------------------------------------------
Chattonella_ovataAF210738           ----------------------------------------------------
Chattonella_marinaAY704162          ----------------------------------------------------
Chattonella_ovataAY704163           ----------------------------------------------------
Vacuolaria_virescensAF210742        ----------------------------------------------------
Vacuolaria_virescensAF409125        ----------------------------------------------------
Heterosigma_akashiwo_AF210741       ----------------------------------------------------
Heterosigma_akashiwoAF086948        ----------------------------------------------------
Heterosigma_akashiwoAY704161        ----------------------------------------------------
Heterosigma_akashiwoAF042820        ----------------------------------------------------
Olisthodiscus_luteusAF210743        ----------------------------------------------------
Cylindrotheca_closteriumAF4176      ----------------------------------------------------

Chattonella_subsalsaAF210736        ----------------------------------------------------
Chattonella_subsalsaAF409126        CCTAGATCCCACAAAAGGTGTTGGTTCATTTAGACAGCAGGACGGTGGCC 748
Chattonella cf. subsalsa CHPI36     CCTAGATCCCACAAAAGGTGTTGGTTCATTTAGACAGCAGGACGGTGGCC 748
Chattonella_antiquaAF210737         ----------------------------------------------------
Chattonella_marinaAF210739          ----------------------------------------------------
Chattonella_ovataAF210738           ----------------------------------------------------
Chattonella_marinaAY704162          ----------------------------------------------------
Chattonella_ovataAY704163           ----------------------------------------------------
Vacuolaria_virescensAF210742        ----------------------------------------------------
Vacuolaria_virescensAF409125        ----------------------------------------------------
Heterosigma_akashiwo_AF210741       ----------------------------------------------------
Heterosigma_akashiwoAF086948        ----------------------------------------------------
Heterosigma_akashiwoAY704161        ----------------------------------------------------
Heterosigma_akashiwoAF042820        ----------------------------------------------------
Olisthodiscus_luteusAF210743        ----------------------------------------------------
Cylindrotheca_closteriumAF4176      ----------------------------------------------------

Chattonella_subsalsaAF210736        ----------------------------------------------------
Chattonella_subsalsaAF409126        ATGGAAGTCGGAACCCGCTAAGGAGTGTGTAACAACTCACCTGCCGAATG 798
Chattonella cf. subsalsa CHPI36     ATGGAAGTCGGAACCCGCTAAGGAGTGTGTAACAACTCACCTGCCGAATG 798
Chattonella_antiquaAF210737         ----------------------------------------------------
Chattonella_marinaAF210739          ----------------------------------------------------
Chattonella_ovataAF210738           ----------------------------------------------------
Chattonella_marinaAY704162          ----------------------------------------------------
Chattonella_ovataAY704163           ----------------------------------------------------
Vacuolaria_virescensAF210742        ----------------------------------------------------
Vacuolaria_virescensAF409125        ----------------------------------------------------
Heterosigma_akashiwo_AF210741       ----------------------------------------------------
Heterosigma_akashiwoAF086948        ----------------------------------------------------
Heterosigma_akashiwoAY704161        ----------------------------------------------------
Heterosigma_akashiwoAF042820        ----------------------------------------------------
Olisthodiscus_luteusAF210743        ----------------------------------------------------
Cylindrotheca_closteriumAF4176      ----------------------------------------------------

Chattonella_subsalsaAF210736        ----------------------------------------------------
Chattonella_subsalsaAF409126        AACTAGCCCTGAAAATGGATGGCGCTAAAGCGTGCGACTTATACTAAGCC 848
Chattonella cf. subsalsa CHPI36     AACTAGCCCTGAAAATGGATGGCGCTAAAGCGTGCGACTTATACTAAGCC 848
Chattonella_antiquaAF210737         ----------------------------------------------------
Chattonella_marinaAF210739          ----------------------------------------------------
Chattonella_ovataAF210738           ----------------------------------------------------
Chattonella_marinaAY704162          ----------------------------------------------------
Chattonella_ovataAY704163           ----------------------------------------------------
Vacuolaria_virescensAF210742        ----------------------------------------------------
Vacuolaria_virescensAF409125        ----------------------------------------------------
Heterosigma_akashiwo_AF210741       ----------------------------------------------------
Heterosigma_akashiwoAF086948        ----------------------------------------------------
Heterosigma_akashiwoAY704161        ----------------------------------------------------
Heterosigma_akashiwoAF042820        ----------------------------------------------------
Olisthodiscus_luteusAF210743        ----------------------------------------------------
Cylindrotheca_closteriumAF4176      ----------------------------------------------------
```

```
Chattonella_subsalsaAF210736          ------------------------------
Chattonella_subsalsaAF409126          ATCAGTGCGAGATGTATGCACTGATGAGTAG 879
Chattonella cf. subsalsa CHPI36       ATCAGTGCGAGACGTATGCACTGATGAGTAG 879
Chattonella_antiquaAF210737           ------------------------------
Chattonella_marinaAF210739            ------------------------------
Chattonella_ovataAF210738             ------------------------------
Chattonella_marinaAY704162            ------------------------------
Chattonella_ovataAY704163             ------------------------------
Vacuolaria_virescensAF210742          ------------------------------
Vacuolaria_virescensAF409125          ------------------------------
Heterosigma_akashiwo_AF210741         ------------------------------
Heterosigma_akashiwoAF086948          ------------------------------
Heterosigma_akashiwoAY704161          ------------------------------
Heterosigma_akashiwoAF042820          ------------------------------
Olisthodiscus_luteusAF210743          ------------------------------
Cylindrotheca_closteriumAF4176        ------------------------------
```

Figure 4. Sequence alignment of ITS-rDNA of raphidophyte species

```
Vacuolaria_virescensAF409125      ----------------------------------------------------
Heterosigma_akashiwoAY858875      ----------------------------------------------------
Chattonella_marinaAY858861        ----------------------------------------------------
Chattonella_marinaAY858862        ----------------------------------------------------
Chattonella_ovataAY858872         ----------------------------------------------------
Chattonella_marinaAY865604        ----------------------------------------------------
Chattonella_marinaAY704165        ---------------------------------ACACCGATCCTAA-CGG 16
Chattonella_ovataAY704166         ---------------------------------ACACCGATCCTAAACGG 17
Chattonella_antiquaAF136761       --------------CTGCGGAAGGATCATTACCACACCGATCCTAAACGG 36
Chattonella_marinaAF137074        --------------CTGCGGAAGGATCATTACCACACCGATCCTAAACGG 36
Chattonella_ovataAY858863         ----------------------------------------------------
Chattonella_subsalsaAF409126      ----------------------------------------------------
Chattonella_subsalsaAY858869      ----------------------------------------------------
Chattonella_subsalsaAF153195      ----------------------------------------------------
Chattonella cf. subsalsaCHPI36    ----------------------------------------------------
Chattonella_subsalsaAY858870      ----------------------------------------------------
Chattonella_subsalsaAY858864      ----------------------------------------------------
Chattonella_subsalsaAY858866      ----------------------------------------------------
Chattonella_subsalsaAY858868      ----------------------------------------------------
Chattonella_subsalsaAY858871      ----------------------------------------------------
Fibrocapsa_japonica               ----------------------------------------------------
Olisthodiscus_luteus              ----------------------------------------------------

Vacuolaria_virescensAF409125      ---------CGTCGTGAAACTTGTTTTTCGTC-CGGC---CCGTCGACAA 37
Heterosigma_akashiwoAY858875      -----------TCGTGAAACTGTTTCCTGGCCTCGGC---CAGTCGACAC 36
Chattonella_marinaAY858861        -----------TCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 36
Chattonella_marinaAY858862        -----------TCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 36
Chattonella_ovataAY858872         -----------TCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 36
Chattonella_marinaAY865604        -----------TCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 36
Chattonella_marinaAY704165        GATCCGTCCCATCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 63
Chattonella_ovataAY704166         GATCCGTCCCATCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 64
Chattonella_antiquaAF136761       GATCCGTCCCATCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 83
Chattonella_marinaAF137074        GATCCGTCCCATCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 83
Chattonella_ovataAY858863         -----------TCGTGAACTTGTTTCCGGGCCTTGGC---CCGTTGACAG 36
Chattonella_subsalsaAF409126      ------------------------------------------TGACAT 6
Chattonella_subsalsaAY858869      ------------------------------------------TGACAT 6
Chattonella_subsalsaAF153195      -----------TCGTGAACTTGTTTCCGGGCTTGGGC---CCGTTGACAT 36
Chattonella cf. subsalsaCHPI36    ------------------------------------------TGACAT 6
Chattonella_subsalsaAY858870      -----------TCGTGAACTTGTTTCCGGGCTTCGGC---CCGTTGACAT 36
Chattonella_subsalsaAY858864      -----------TCGTGAACTTGTTTCCGGGCTTCGGC---CCGTTGACAT 36
Chattonella_subsalsaAY858866      -----------TCGTGAACTTGTTTCCGGGCTTCGGC---CCGTTGACAT 36
Chattonella_subsalsaAY858868      -----------TCGTGAACTTGTTTCCGGGCTTCGGC---CCGTTGACAT 36
Chattonella_subsalsaAY858871      -----------TCGTGAACTTGTTTCCGGGCTTCGGC---CCGTTGACAT 36
Fibrocapsa_japonica               ----AGTTTTTCCGCTGATCCACGGTTTAACCGTACT--TGTACGGTGAA 44
Olisthodiscus_luteus              --------AGGTAGCGAGGATTATTCCCCTACCTGAC---TCCCAAAAAA 39
                                                                            *

Vacuolaria_virescensAF409125      CTTTGA-ACCAACCAAACC-AAACC-TCAAAACCCAAAAATTTTGACCCA 84
Heterosigma_akashiwoAY858875      TTCATA-ACCAAAC---CC-AAACC-TCAAAACC--AAAGTTTTGACCCA 78
Chattonella_marinaAY858861        CTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 83
Chattonella_marinaAY858862        CTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 83
Chattonella_ovataAY858872         CTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 83
Chattonella_marinaAY865604        MTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 83
Chattonella_marinaAY704165        CTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 110
Chattonella_ovataAY704166         CTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 111
Chattonella_antiquaAF136761       CTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 130
Chattonella_marinaAF137074        CTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 130
Chattonella_ovataAY858863         CTTTTACACCCATCCAACC-AAACC-TCAAAACC-AAACATTTTGACCCT 83
Chattonella_subsalsaAF409126      CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 54
Chattonella_subsalsaAY858869      CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 54
Chattonella_subsalsaAF153195      CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 84
Chattonella cf. subsalsaCHPI36    CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 54
Chattonella_subsalsaAY858870      CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 84
Chattonella_subsalsaAY858864      CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 84
Chattonella_subsalsaAY858866      CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 84
Chattonella_subsalsaAY858868      CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 84
Chattonella_subsalsaAY858871      CCTTTACACCCATCCAACCCAAACC-TCAAAACC-AAAAATTTTGACCCA 84
Fibrocapsa_japonica               GCCAAACCCCATTTTAACCCAAGC--TGAATTTTGAAAGGGCGAGGGAAA 92
Olisthodiscus_luteus              CC---ACGAATACTTACCGTACAAC-CCAAAACC-CTTTACTCCTATTTT 84
                                       *           * *       **

Vacuolaria_virescensAF409125      TCCAACCCATCCGACTGAACTGAAACGGCG-GCCTTTCCCTGTTCATCCG 133
Heterosigma_akashiwoAY858875      AACCAACCTCCAATCTGAACCGTAACGGGA--------------CGGCCG 114
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Chattonella_marinaAY858861        ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 121
Chattonella_marinaAY858862        ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 121
Chattonella_ovataAY858872         ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 121
Chattonella_marinaAY865604        ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 121
Chattonella_marinaAY704165        ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 148
Chattonella_ovataAY704166         ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 149
Chattonella_antiquaAF136761       ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 168
Chattonella_marinaAF137074        ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 168
Chattonella_ovataAY858863         ACCAACCCATTCGACTGAACCGTAACGGTT-GCTT-----------CTCT 121
Chattonella_subsalsaAF409126      ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCCT 92
Chattonella_subsalsaAY858869      ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCCT 92
Chattonella_subsalsaAF153195      ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCCT 122
Chattonella cf. subsalsaCHPI36    ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCTT 92
Chattonella_subsalsaAY858870      ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCCT 122
Chattonella_subsalsaAY858864      ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCCT 122
Chattonella_subsalsaAY858866      ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCCT 122
Chattonella_subsalsaAY858868      ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCCT 122
Chattonella_subsalsaAY858871      ACCAACCCATTCGACTGAACCGTAACGGCC-GCTT-----------CCCT 122
Fibrocapsa_japonica               GGGTCCTCATACCTTAGTACTTTGTCCTC----------------CCATT 126
Olisthodiscus_luteus              AACAACCTAATCTAAAGAAACTTCTAGACT-AGTGATGGTGGGTTCTCTG 133
                                        * *

Vacuolaria_virescensAF409125      GGGAAAGCCGCCGGCCAATTTC-AAAA----CAAAGTCA--TACGACTTT 176
Heterosigma_akashiwoAY858875      C--AAGGCCTCCCGACGATTAT-CTAA----TAA--TCA--TACGACTTT 153
Chattonella_marinaAY858861        --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 158
Chattonella_marinaAY858862        --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 158
Chattonella_ovataAY858872         --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 158
Chattonella_marinaAY865604        --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 158
Chattonella_marinaAY704165        --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 185
Chattonella_ovataAY704166         --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 186
Chattonella_antiquaAF136761       --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 205
Chattonella_marinaAF137074        --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 205
Chattonella_ovataAY858863         --GGAAGCAATCGG-CGATTT--AAA-----CAAA-TCA--TACGACTTT 158
Chattonella_subsalsaAF409126      CGGGAGGCAGCCGA-CGATTT--ATAT----CAAA-TCA--TACGACTTT 132
Chattonella_subsalsaAY858869      CGGGAGGCAGCCGA-CGATTT--ATAT----CAAA-TCA--TACGACTTT 132
Chattonella_subsalsaAF153195      CGGGAGGCAGCCGA-CGATTT--ATAT----CAAA-TCA--TACGACTTT 162
Chattonella cf. subsalsaCHPI36    CGGGAGGCAGCCGA-CGATTT--ACAT----CAAA-TCA--TACGACTTT 132
Chattonella_subsalsaAY858870      CGGGAGGCAGCCGA-CGATTT--ATAT----CAAA-TCA--TACGACTTT 162
Chattonella_subsalsaAY858864      CGGGAGGCAGCCGA-CGATTT--ATAT----CAAA-TCA--TACGACTTT 162
Chattonella_subsalsaAY858866      CGGGAGGCAGCCGA-CGATTT--ATAT----CAAA-TCA--TACGACTTT 162
Chattonella_subsalsaAY858868      CGGGAGGCAGCCGA-CGATTT--ATAT----CAAA-TCA--TACGACTTT 162
Chattonella_subsalsaAY858871      CGGGAGGCAGCCGA-CGATTT--ATAT----CAAA-TCA--TACGACTTT 162
Fibrocapsa_japonica               GAGCGGTCAGCCGTGAGGTTGGTCGGA----TAATTTAAAATATAACTTT 172
Olisthodiscus_luteus              CCATTGCCGGTTTACCAATTAC-CAAA----TTAT-CCA--TA--ACTTT 173
                                         *          **             *    *  **  *****

Vacuolaria_virescensAF409125      CAGCAATGGATGTCTTGGCTCCCACAACGATGAAGAACGCAGCGAAATGC 226
Heterosigma_akashiwoAY858875      CAGCGGCGGATGTCTTGGTTCCCACATCGATGAAGAACGCAGCGAAATGC 203
Chattonella_marinaAY858861        CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 208
Chattonella_marinaAY858862        CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 208
Chattonella_ovataAY858872         CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 208
Chattonella_marinaAY865604        CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 208
Chattonella_marinaAY704165        CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 235
Chattonella_ovataAY704166         CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 236
Chattonella_antiquaAF136761       CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 255
Chattonella_marinaAF137074        CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 255
Chattonella_ovataAY858863         CAGCAGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 208
Chattonella_subsalsaAF409126      CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 182
Chattonella_subsalsaAY858869      CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 182
Chattonella_subsalsaAF153195      CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 212
Chattonella cf. subsalsaCHPI36    CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 182
Chattonella_subsalsaAY858870      CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 212
Chattonella_subsalsaAY858864      CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 212
Chattonella_subsalsaAY858866      CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 212
Chattonella_subsalsaAY858868      CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 212
Chattonella_subsalsaAY858871      CAGCGGCGGATGTCTTGGTTCCCACAACGATGAAGAACGCAGCGAAATGC 212
Fibrocapsa_japonica               CAGCAATGGATGTCTTGGCTCCCACAACGATGAAGAACGCAGCGAAATGC 222
Olisthodiscus_luteus              CAGCAACGGATGTCTTGGCTCCTACAACGATGAAGAACGCAGCGAAATGC 223
                                  ****   *********** *** *** ************************

Vacuolaria_virescensAF409125      GATACGTAA-GCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 275
Heterosigma_akashiwoAY858875      GATACGTAATGCGAATTGCAGAGTCTTGCGAGTCATCAAATTTTCGAACG 253
Chattonella_marinaAY858861        GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 258
Chattonella_marinaAY858862        GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 258
Chattonella_ovataAY858872         GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 258
Chattonella_marinaAY865604        GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 258
Chattonella_marinaAY704165        GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 285
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Chattonella_ovataAY704166          GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 286
Chattonella_antiquaAF136761        GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 305
Chattonella_marinaAF137074         GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 305
Chattonella_ovataAY858863          GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 258
Chattonella_subsalsaAF409126       GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 232
Chattonella_subsalsaAY858869       GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 232
Chattonella_subsalsaAF153195       GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 262
Chattonella_subsalsaCHPI36_        GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 232
Chattonella_subsalsaAY858870       GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 262
Chattonella_subsalsaAY858864       GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 262
Chattonella_subsalsaAY858866       GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 262
Chattonella_subsalsaAY858868       GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 262
Chattonella_subsalsaAY858871       GATACGTAATGCGAATTGCAGAGTCCAGCGAGTCATCAAATGTTCGAACG 262
Fibrocapsa_japonica                GATACGTCATGCGAATTGCAGAGTCCAGCGAGTCATCAAAATTTCGAACG 272
Olisthodiscus_luteus               GATACGTAATGCGAATTGCAGAGTCTAGTGAATCATCAAAATTTTGAACG 273
                                   ******* * ***************  * ** ********  ** *****

Vacuolaria_virescensAF409125       CACCTGGCGCTTTCGGGATATTCCTGGAAGCATGCTTGTTAGAGTGTCTG 325
Heterosigma_akashiwoAY858875       CACCTGGCACTTCCGGG-TATTCCTGGGAGTATGCTTTATAGAGTGTCTG 302
Chattonella_marinaAY858861         CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 308
Chattonella_marinaAY858862         CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 308
Chattonella_ovataAY858872          CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 308
Chattonella_marinaAY865604         CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 308
Chattonella_marinaAY704165         CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 335
Chattonella_ovataAY704166          CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 336
Chattonella_antiquaAF136761        CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 355
Chattonella_marinaAF137074         CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 355
Chattonella_ovataAY858863          CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 308
Chattonella_subsalsaAF409126       CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTGGAGTGTCTG 282
Chattonella_subsalsaAY858869       CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTGGAGTGTCTG 282
Chattonella_subsalsaAF153195       CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTGGAGTGTCTG 312
Chattonella cf. subsalsaCHPI36     CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTAGAGTGTCTG 282
Chattonella_subsalsaAY858870       CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTGGAGTGTCTG 312
Chattonella_subsalsaAY858864       CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTGGAGTGTCTG 312
Chattonella_subsalsaAY858866       CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTGGAGTGTCTG 312
Chattonella_subsalsaAY858868       CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTGGAGTGTCTG 312
Chattonella_subsalsaAY858871       CACCTGGCACTTCCGGGATATTCCTGGGAGTATGCTTGTTGGAGTGTCTG 312
Fibrocapsa_japonica                CACCTGGCGCTTCCGGGCATCCCCTGGGAGCATGCTTGTTTGAGTGTCCG 322
Olisthodiscus_luteus               CACATTGCGCTTCC-------TTCTGGGAGCATGTCTTTTGGAGTGTCTT 316
                                   *** * ** *** *         **** ** ***  *  * *******

Vacuolaria_virescensAF409125       TTGG---ATCA-TCTCCCCCCTTTTCCTGCGGGATTAGCGGGCGGCGGAG 371
Heterosigma_akashiwoAY858875       TTGG---ACCA-TCACCCCC-TTTTCCCCCGGGACGA--GGGCGGCGGAG 345
Chattonella_marinaAY858861         TTGG---ACCA-TCTCCCTTGTTCCT--TCGGGAAGA---AGTGGCGGTA 349
Chattonella_marinaAY858862         TTGG---ACCA-TCTCCCTTGTTCCT--TCGGGAAGA---AGTGGCGGTA 349
Chattonella_ovataAY858872          TTGG---ACCA-TCTCCCTTGTTCCT--TCGGGAAGA---AGTGGCGGTA 349
Chattonella_marinaAY865604         TTGG---ACCA-TCTCCCTTGTTCCT--TCGGGAAGA---AGTGGCGGTA 349
Chattonella_marinaAY704165         TTGG---ACCA-TCTCCCTTGTTCCT--TCGGGAAGA---AGTGGCGGTA 376
Chattonella_ovataAY704166          TTGG---ACCA-TCTCCCTTGTTCCT--TCGGGAAGA---AGTGGCGGTA 377
Chattonella_antiquaAF136761        TTGG---ACCA-TCTCCCTTGTTCCT--TTGGGAAGA---AGTGGCGGTA 396
Chattonella_marinaAF137074         TTGG---ACCA-TCTCCCTTGTTCCT--TCGGGAAGA---AGTGGCGGTA 396
Chattonella_ovataAY858863          TTGG---ACCA-TCTCCCTTGTTCCT--TCGGGAAGA---AGTGGCGGTA 349
Chattonella_subsalsaAF409126       TTGG---ACCA-TCTCCCTCGTTCCCACTGGGCAACG---AGTGGCGGAG 325
Chattonella_subsalsaAY858869       TTGG---ACCA-TCTCCCTCGTTCCCACTGGGCAACG---AGTGGCGGAG 325
Chattonella_subsalsaAF153195       TTGG---ACCA-TCTCCCTCGTTCCCACTGGGCAACG---AGTGGCGGAG 355
Chattonella cf. subsalsaCHPI36     TTGG---ACCA-TCTCCCTCGTTCCCACTGGGTAACG---AGCGGCGGAG 325
Chattonella_subsalsaAY858870       TTGG---ACCA-TCTCCCTCGTTCCCACTGGGCAACG---AGTGGCGGAG 355
Chattonella_subsalsaAY858864       TTGG---ACCA-TCTCCCTCGTTCCCACTGGGCAACG---AGTGGCGGAG 355
Chattonella_subsalsaAY858866       TTGG---ACCA-TCTCCCTCGTTCCCACTGGGCAACG---AGTGGCGGAG 355
Chattonella_subsalsaAY858868       TTGG---ACCA-TCTCCCTCGTTCCCACTGGGCAACG---AGTGGCGGAG 355
Chattonella_subsalsaAY858871       TTGG---ACCA-TCTCCCTCGTTCCCACTGGGCAACG---AGTGGCGGAG 355
Fibrocapsa_japonica                TTAGTGTACCGCTCACCCCGTTATTTTGACGGC----------TGGTGGTG 363
Olisthodiscus_luteus               TTAA---AATA-TCTCACCAACTTCCACGGTGGAATA--------TGTCT 354
                                   **     *    ** * *             *              *

Vacuolaria_virescensAF409125       GTTGCTGTCCACCGCC-TCGTTT---TGCGGTGCTCAGTGAAAATTTCAA 417
Heterosigma_akashiwoAY858875       GTTGCTCGCCACTTCT-CTTCGG---AGAGGTGATGAGTCAAGCACTCGA 391
Chattonella_marinaAY858861         GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 398
Chattonella_marinaAY858862         GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 398
Chattonella_ovataAY858872          GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 398
Chattonella_marinaAY865604         GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 398
Chattonella_marinaAY704165         GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 425
Chattonella_ovataAY704166          GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 426
Chattonella_antiquaAF136761        GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 445
Chattonella_marinaAF137074         GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 445
Chattonella_ovataAY858863          GTTGCCGTACATTTTG-CTCTTTGAGCGAGATGATCGGTTAAGCACTCGA 398
Chattonella_subsalsaAF409126       GTTGCCGTACATTTTG-CTCCCTGAGCGGGATGATCGGTTAAGCACTCGA 374
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Chattonella_subsalsaAY858869      GTTGCCGTACATTTTG-CTCCCTGAGCGGGATGATCGGTTAAGCACTCGA 374
Chattonella_subsalsaAF153195      GTTGCCGTACATTTTG-CTCCCTGAGCGGGATGATCGGTTAAGCACTCGA 404
Chattonella cf. subsalsaCHPI36    GTTGCCGTACATTTTG-CTCTTCGAGTGGGATGATCGGTGAAGCACTCGA 374
Chattonella_subsalsaAY858870      GTTGCCGTACATTTTG-CTCCCTGAGCGGGATGATCGGTTAAGCACTCGA 404
Chattonella_subsalsaAY858864      GTTGCCGTACATTTTG-CTCCCTGAGCGGGATGATCGGTTAAGCACTCGA 404
Chattonella_subsalsaAY858866      GTTGCCGTACATTTTG-CTCCCTGAGCGGGATGATCGGTTAAGCACTCGA 404
Chattonella_subsalsaAY858868      GTTGCCGTACATTTTG-CTCCCTGAGCGGGATGATCGGTTAAGCACTCGA 404
Chattonella_subsalsaAY858871      GTTGCCGTACATTTTG-CTCCCTGAGCGGGATGATCGGTTAAGCACTCGA 404
Fibrocapsa_japonica               TTTGCCTTCCACCCACACTCCTAAAGTGTGGCTGTCTGGCGTG-GTCTGG 412
Olisthodiscus_luteus              TTCACTGCTCCTTTAC-ATGGGGTAGTGTAG-GATTAAATACGAACCTTT 402
                                   *  *    *                 *      *

Vacuolaria_virescensAF409125      CATGGCAGCCCCGGCCTACCGGCG-GGTGTGGTAGCGCTTGGCCCTTGCG 466
Heterosigma_akashiwoAY858875      GATACAAACCGTCGAG-ACCGACG-GGCGTGGTAGCGAT----CTTCACG 435
Chattonella_marinaAY858861        GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 445
Chattonella_marinaAY858862        GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 445
Chattonella_ovataAY858872         GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 445
Chattonella_marinaAY865604        GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 445
Chattonella_marinaAY704165        GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 472
Chattonella_ovataAY704166         GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 473
Chattonella_antiquaAF136761       GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 492
Chattonella_marinaAF137074        GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 492
Chattonella_ovataAY858863         GATAACAACCGTTGAGTTC-AACG-GGCGTGGTAGCGCTGGG-CCTCATG 445
Chattonella_subsalsaAF409126      GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 420
Chattonella_subsalsaAY858869      GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 420
Chattonella_subsalsaAF153195      GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 450
Chattonella cf. subsalsaCHPI36    GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 420
Chattonella_subsalsaAY858870      GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 450
Chattonella_subsalsaAY858864      GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 450
Chattonella_subsalsaAY858866      GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 450
Chattonella_subsalsaAY858868      GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 450
Chattonella_subsalsaAY858871      GATAACAACCGTTGGATTCCGACG-GGCGTGGTAGCGCTGGG-CTTC--G 450
Fibrocapsa_japonica               GATATTACCTCCCCACCCC------GGCTGTATAGCATTGCTGTATGTTG 456
Olisthodiscus_luteus              AACGGC--TCACTAGCTTCTTTCGAGGCAGAATAACACTCGG-TTATAAA 449
                                   *                *      **     ** *  *

Vacuolaria_virescensAF409125      GCAAAGCTCACAGCCTCCCCGATGGCAAAGGTCGGAGCTCGTCCGATTTC 516
Heterosigma_akashiwoAY858875      ATCA----CACATCCTCCCCGGCGA--------GAACTTCGGTTG-TTCT 472
Chattonella_marinaAY858861        GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCGGTTGATCT- 486
Chattonella_marinaAY858862        GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCGGTTGATCT- 486
Chattonella_ovataAY858872         GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCGGTTGATCT- 486
Chattonella_marinaAY865604        GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCGGTTGATCT- 486
Chattonella_marinaAY704165        GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCGGTTGATCT- 513
Chattonella_ovataAY704166         GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCCGTTGATCT- 514
Chattonella_antiquaAF136761       GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCGGTTGATCT- 533
Chattonella_marinaAF137074        GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCGGTTGATCT- 533
Chattonella_ovataAY858863         GCTAAGCACAAATCCTCCCCGGTAG--------AAGTCTCGGTTGATCT- 486
Chattonella_subsalsaAF409126      GCCAAGCACACATCCTCCCCGGCAG--------AAACCTCGGTTGATCT- 461
Chattonella_subsalsaAY858869      GCCAAGCACACATCCTCCCCGGCAG--------AAACCTCGGTTGATCT- 461
Chattonella_subsalsaAF153195      GCCAAGCACACATCCTCCCCGGCAG--------AAACCTCGGTTGATCT- 491
Chattonella cf. subsalsaCHPI36    GCCAAGCACACATCCTCCCCGACAG--------AAACCTCGGTTGATCT- 461
Chattonella_subsalsaAY858870      GCCAAGCACACATCCTCCCCGGCAG--------AAACCTCGGTTGATCT- 491
Chattonella_subsalsaAY858864      GCCAAGCACACATCCTCCCCGGCAG--------AAACCTCGGTTGATCT- 491
Chattonella_subsalsaAY858866      GCCAAGCACACATCCTCCCCGGCAG--------AAACCTCGGTTGATCT- 491
Chattonella_subsalsaAY858868      GCCAAGCACACATCCTCCCCGGCAG--------AAACCTCGGTTGATCT- 491
Chattonella_subsalsaAY858871      GCCAAGCACACATCCTCCCCGGCAG--------AAACCTCGGTTGATCT- 491
Fibrocapsa_japonica               GCATGTTGTGTTATGGTCCGGTTGTGCCCGTCCCATTCGTGGTTGATGGG 506
Olisthodiscus_luteus              GATCGGTATAGTTTGATCCCCGCGT--------GAGGTTGAGCCGCTTGT 491
                                                  **                      * *

Vacuolaria_virescensAF409125      AACCGAGGAAACAAGGGTAGGAAAGGCGGGCCCTCCGGGACCCCCCCGAC 566
Heterosigma_akashiwoAY858875      GACAGAGGAA------------------------CTGGTGTTGCTTCGGC 498
Chattonella_marinaAY858861        AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 512
Chattonella_marinaAY858862        AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 512
Chattonella_ovataAY858872         AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 512
Chattonella_marinaAY865604        AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 512
Chattonella_marinaAY704165        AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 539
Chattonella_ovataAY704166         AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 540
Chattonella_antiquaAF136761       AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 559
Chattonella_marinaAF137074        AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 559
Chattonella_ovataAY858863         AACAGAGGAA------------------------CTGGAAGAAGCTTCGG 512
Chattonella_subsalsaAF409126      GACAGAG-AA------------------------CTGGGTGG-------- 478
Chattonella_subsalsaAY858869      GACAGAGGAA------------------------CTGGGTGGCTT----- 482
Chattonella_subsalsaAF153195      GACAGAGGAA------------------------CTGGGTGGCTT----- 512
Chattonella cf. subsalsaCHPI36    GACAGAGGAT------------------------CTGG-TGG-------- 478
Chattonella_subsalsaAY858870      GACAGAGGAA---------------------------------------- 501
Chattonella_subsalsaAY858864      GACAGAGGAA---------------------------------------- 501
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Chattonella_subsalsaAY858866      GACAGAGGAA---------------------------------------- 501
Chattonella_subsalsaAY858868      GACAGAGGAA---------------------------------------- 501
Chattonella_subsalsaAY858871      GACAGAGGAA---------------------------------------- 501
Fibrocapsa_japonica               GCACGAGGTGGTA----TACAAAGATAAGACGTGCTGCCATTTTTGGTGT 552
Olisthodiscus_luteus              AGATGTGGTAGTA----TCGGATTTGTCCTTCGGGACGAAAACGTGCGGA 537
                                      * *

Vacuolaria_virescensAF409125      TTGCCCCCACATTTCAGATCTCTAATCAAGCAAGAAGACCCGCTGAATTT 616
Heterosigma_akashiwoAY858875      AT---CCCACTGTTCAGATCTCTATTGAAGCAAGAAGACCCCCTGAATTT 545
Chattonella_marinaAY858861        CTGAATCCACTTTTCAGATCTCTAATCAAGCAAGAAGACCCGCTGAATTT 562
Chattonella_marinaAY858862        CTGAATCCACTTTTCAGATCTCTAATCAAGCAAGAAGACCCGCTGAATTT 562
Chattonella_ovataAY858872         CTGAATCCACTTTTCAGATCTCTAATCAAGCAAGAAGACCCGCTGAATTT 562
Chattonella_marinaAY865604        CTGAATCCACTTTTCAGATCTCTAATCAAGCAAGAAGACCCGCTGAATTT 562
Chattonella_marinaAY704165        CTGAATCCACTTTTCAGATCTCTATCAA-GCAAGAA-------------- 574
Chattonella_ovataAY704166         CTGAATCCACTTTTCAGATCTCTAATCA-GCAGAAG------------- 575
Chattonella_antiquaAF136761       CTGAATCCACTTTTCAGATCTCTAATCAAGCAAGAAGACCCGCTGAATTT 609
Chattonella_marinaAF137074        CTGAATCCACTTTTCAGATCTCTAATCAAGCAAGAAGACCCGCTGAATTT 609
Chattonella_ovataAY858863         CTGAATCCACTTTTCAGATCTCTAATCAAGCAAGAAGACCCGCTGAA--- 559
Chattonella_subsalsaAF409126      ---------------------------------------------------
Chattonella_subsalsaAY858869      ---------------------------------------------------
Chattonella_subsalsaAF153195      ---------------------------------------------------
Chattonella cf. subsalsaCHPI36    ---------------------------------------------------
Chattonella_subsalsaAY858870      ---------------------------------------------------
Chattonella_subsalsaAY858864      ---------------------------------------------------
Chattonella_subsalsaAY858866      ---------------------------------------------------
Chattonella_subsalsaAY858868      ---------------------------------------------------
Chattonella_subsalsaAY858871      ---------------------------------------------------
Fibrocapsa_japonica               GCTAGTCTTGTCTGCCACTTTGTGTCAGTTGCAATCGGTTTTCCGTAGCT 602
Olisthodiscus_luteus              TCATTTACTGAAGCCTGGTTCCTGATCGTGGTGCGGGTCTTTCT-ATTTC 586

Vacuolaria_virescensAF409125      AAGCATATTAATAAGCGGAGGAAAAGAAACC------------------- 647
Heterosigma_akashiwoAY858875      AAGCATAT------------------------------------------ 553
Chattonella_marinaAY858861        AAGCATAT------------------------------------------ 570
Chattonella_marinaAY858862        AAGCATAT------------------------------------------ 570
Chattonella_ovataAY858872         AAGCATAT------------------------------------------ 570
Chattonella_marinaAY865604        AAGCATAT------------------------------------------ 570
Chattonella_marinaAY704165        ---------------------------------------------------
Chattonella_ovataAY704166         ---------------------------------------------------
Chattonella_antiquaAF136761       AA------------------------------------------------ 611
Chattonella_marinaAF137074        AAGCCAATCAATA------------------------------------- 622
Chattonella_ovataAY858863         ---------------------------------------------------
Chattonella_subsalsaAF409126      ---------------------------------------------------
Chattonella_subsalsaAY858869      ---------------------------------------------------
Chattonella_subsalsaAF153195      ---------------------------------------------------
Chattonella cf. subsalsaCHPI36    ---------------------------------------------------
Chattonella_subsalsaAY858870      ---------------------------------------------------
Chattonella_subsalsaAY858864      ---------------------------------------------------
Chattonella_subsalsaAY858866      ---------------------------------------------------
Chattonella_subsalsaAY858868      ---------------------------------------------------
Chattonella_subsalsaAY858871      ---------------------------------------------------
Fibrocapsa_japonica               GCACGTGTTGATCTCTATAGTATTTGTGTGCATGCTTAGCTGGATGGGTG 652
Olisthodiscus_luteus              AGGACAATTCCCCTGTGCTGTATTAGAATCG------------------- 617

Vacuolaria_virescensAF409125      ------
Heterosigma_akashiwoAY858875      ------
Chattonella_marinaAY858861        ------
Chattonella_marinaAY858862        ------
Chattonella_ovataAY858872         ------
Chattonella_marinaAY865604        ------
Chattonella_marinaAY704165        ------
Chattonella_ovataAY704166         ------
Chattonella_antiquaAF136761       ------
Chattonella_marinaAF137074        ------
Chattonella_ovataAY858863         ------
Chattonella_subsalsaAF409126      ------
Chattonella_subsalsaAY858869      ------
Chattonella_subsalsaAF153195      ------
Chattonella cf. subsalsaCHPI36    ------
Chattonella_subsalsaAY858870      ------
Chattonella_subsalsaAY858864      ------
Chattonella_subsalsaAY858866      ------
Chattonella_subsalsaAY858868      ------
Chattonella_subsalsaAY858871      ------
Fibrocapsa_japonica               TGGCTT 658
Olisthodiscus_luteus              ------
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